

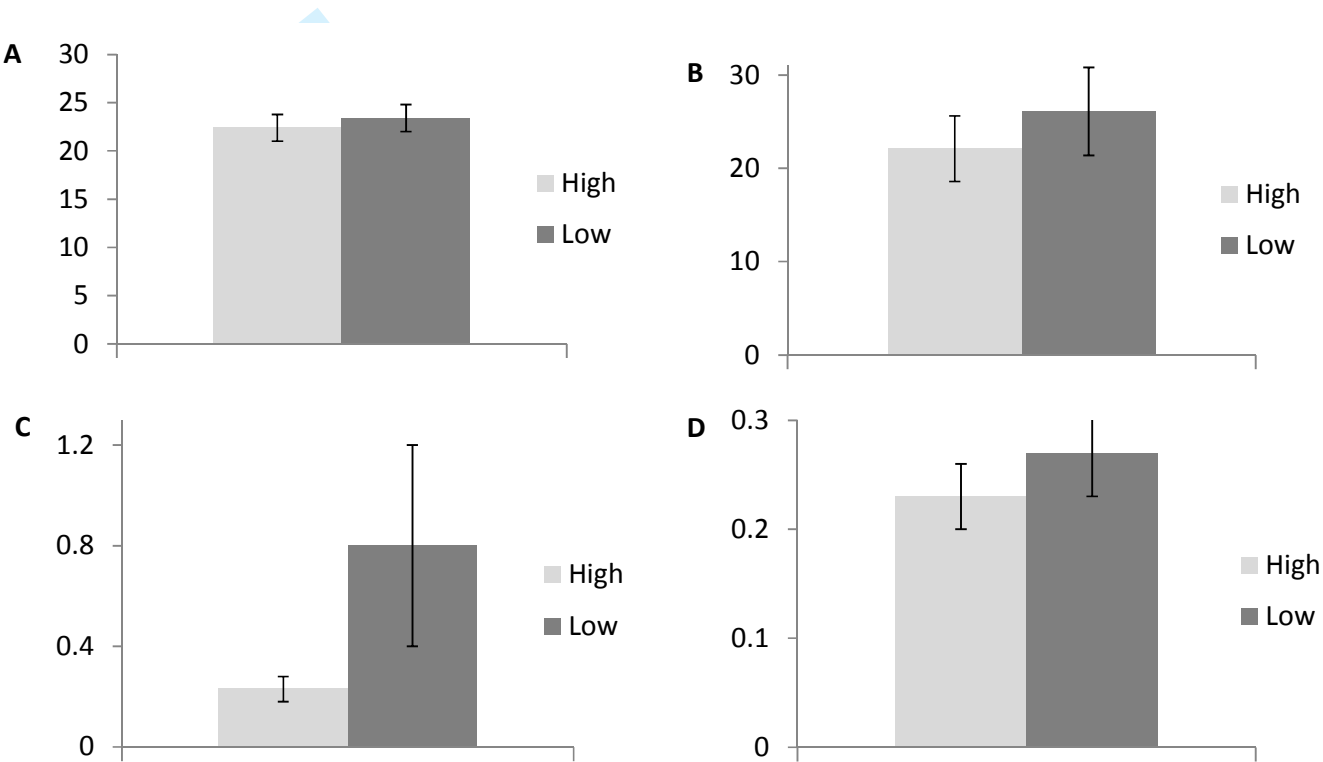
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The association between objectively measured sitting and standing with body composition: A pilot study using magnetic resonance imaging

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Figure 1: comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes



A, Body mass index; B, Total adipose fat (litres); C, liver fat (%); D, visceral/subcutaneous abdominal fat ratio; n=12

The association between objectively measured sitting and standing with body composition: A pilot study using magnetic resonance imaging

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30 **ABSTRACT**

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32 **Objective:** To investigate the association between objectively measured sitting and standing, using a
33 postural allocation technique, with MRI assessed body composition.

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35 **Design:** The present study was a cross-sectional pilot study.

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37 **Setting:** Participants were examined at one centre located in London, UK.

38

39 **Participants:** Normal weight Caucasian females (30.9 ± 6.1 yrs; BMI, 22.9 ± 3.4 kg/m²) with desk
40 bound occupations were sort to minimise variability in body composition outcomes. A convenience
41 sample of 12 females were recruited in January 2014 from University College London

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43 **Outcome measures:** For each participant a number of body composition variables were attained
44 from a single whole-body magnetic resonance imaging session. Main outcome variables included:
45 total and liver adiposity, visceral/subcutaneous fat ratio, and body mass index. Main exposure
46 variables included: average sitting time, standing: sitting ratio, and step count. Pearson Correlations
47 were carried out to examine associations between different activity categories and body
48 composition variables.

49

50 **Results:** There were significant correlations between average daily sitting and liver adiposity and
51 visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively); standing: sitting ratio
52 was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-$
53 0.53 and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total
54 adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively).

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56 **Conclusion:** This pilot study has provided preliminary evidence of relationships between objectively
57 measured sitting and standing and precise measures of body composition.

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Article Summary: strengths and limitations of this study

- This is the first study to show an association between objectively measured sitting and standing, using a postural allocation technique, with MRI assessed body composition.
- The data collection protocol and tools used within this pilot study are feasible and can be implemented into subsequent experimental trials.
- It was not feasible to make multiple statistical adjustments in our analyses owing to the small sample size.

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87 **INTRODUCTION**

88 There is a growing body of literature that suggests sedentary behaviours – defined as any waking
89 behaviour characterised by energy expenditure below 1.5 metabolic equivalents while in a sitting or
90 reclined posture – are associated with higher risk of cardiovascular disease (CVD) and mortality, after
91 statistical adjustment for moderate-to-vigorous intensity physical activities (MVPA; e.g. brisk
92 walking).[1] This has large public health relevance in light of objective data from general adult
93 population studies in the USA and Great Britain that show on average adults spend approximately 60
94 to 70% of their waking hours in sedentary activities.[2] Indeed, westernised society is geared
95 towards promoting sedentary lifestyles (i.e. screen based entertainment, motorised transport etc.),
96 thus, developing strategies to combat sedentary behaviour are crucial.

97 Such a strategy might not necessarily involve exercise of moderate or vigorous intensity, as
98 interventions to increase exercise levels have proved challenging and largely unsuccessful.[3, 4]
99 Instead, given the barriers to structured exercise (e.g. motivation, cost, access and time etc.), we
100 might consider more subtle lifestyle approaches that are primarily designed to displace sedentary
101 behaviour (i.e. sitting) with forms of lighter intensity (incidental) activity (e.g. standing). If population
102 activity patterns can be shifted from predominantly sedentary to the next lowest physical activity
103 (PA) category (standing), consequent interventions targeting moderate or vigorous exercise may
104 then be more successful as it reflects a natural shift along the activity continuum.

105 To date, limited epidemiological evidence has been generated on the associations between light
106 intensity activity and health. This is partially owing to measurement issues; self-reported PA
107 questionnaires are designed to capture MVPA and there are technical limitations in differentiating
108 between sitting, standing and other forms of light activity when interpreting objective activity data.
109 The most commonly used accelerometer, the Actigraph, quantifies time spent in different intensities
110 of activity by summing time above and below specified count thresholds. This method works
111 reasonably well for identifying MVPA, but is less accurate for distinguishing *between* sedentary and
112 light activity (i.e. between sitting and standing).[5] Thus, methods that employ postural allocation
113 may be more reliable, which have only recently become readily available.

114 Some experimental evidence is beginning to emerge in this area. For example, one study
115 manipulated sitting time and PA over one day under free living conditions. The results indicated that
116 replacing sitting with longer periods of light activity was more beneficial for metabolic health than
117 one hour of vigorous exercise despite equivalent daily energy expenditure in each treatment
118 group.[6] In a laboratory controlled trial conducted over an 8 hour period, interrupting sitting time

every 20 mins with short 2-min bouts of light- or moderate intensity walking was shown to lower postprandial glucose and insulin levels in overweight/ obese adults.[7] In another study, using continuously monitored capillary blood glucose, there was a 43% reduction in blood glucose excursion during an afternoon (185 minutes) of standing compared with sitting in desk-based workers.[8] In a pilot study replacing sitting workstations with sit-stand workstations employees reduced sitting time by 137 min/d and increases in HDL-cholesterol were observed at 3 months follow-up.[9] However, the biological mechanisms underlying these effects still remain unclear, although increased muscle activation during standing could be an important underlying mechanism, for example, by increasing skeletal muscle metabolism. Replacing a sitting workstation with a standing workstation was shown to increase daily energy expenditure,[8] thus the longer term benefits might also include reductions in total, visceral, and liver adiposity. A reduction in total and visceral adiposity is known to have a favourable impact on a range of CVD risk factors including inflammatory markers, lipids, and glycaemic control. Liver adiposity is of particular interest as it has been linked to metabolic risk and worsening insulin resistance.[10] Several exercise training studies have demonstrated reductions in visceral adiposity in the absence of weight loss.[11] However, the relationship between light PA (standing) and total, visceral, and liver adiposity has yet to be investigated using precise imaging techniques.

Further research is needed to aid in the understanding of the relationships between objectively measuring sitting and standing, using an objective postural allocation technique, and measures of total, visceral and liver adiposity, using precise imaging techniques. This will contribute to the small but growing body of literature that aims to inform policy and intervention on the health benefits of displacing sitting with standing.

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142 AIM

143 In the present study, several contemporary methods were used, including an objective postural
144 allocation technique in combination with magnetic resonance imaging (MRI), to aid in the
145 understanding of the relationships between sitting/ standing and body composition. This data
146 collection was primarily designed to inform a large experimental trial that will investigate the impact
147 of displacing sitting with standing on total, visceral, and liver adiposity. This pilot data will (i) inform
148 the underlying rationale of the trial by producing evidence, if it exists, of relationships between
149 objective measures of sitting and standing and body composition, and (ii) generate an effect size on
150 which to base sample size calculations to inform the main trial.

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151 **METHODS**

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153 **Design, participants and sample size**

154 This cross-sectional pilot study was carried out in 12 healthy Caucasian females. The sample size for
155 this pilot was based on previous published work, which has shown that significant differences in
156 body composition could be readily observed in cross-sectional studies of 10 or less volunteers.[12]
157 Normal weight females with desk bound occupations were selected from a larger cohort to minimise
158 variability in age, weight and overall anthropometry. A convenience sample was recruited in January
159 2014 from University College London. We randomly invited 12 females who met our criteria to take
160 part in the study. All females invited agreed to take part. One week prior to data collection trained
161 research staff met with the participants to administer the participant information sheets and explain
162 the study protocol.

163

164 **Measures of adiposity**

165 For each participant, a range of body composition variables were attained from a single whole-body
166 MRI session lasting approximately 45 mins. For the purpose of the present study we defined our
167 main outcomes as *a priori*, which included body mass index (BMI), total litres of body adiposity (L),
168 liver adiposity (%), and visceral/subcutaneous abdominal fat ratio. Whole-body MR images and liver
169 adiposity (%) were obtained on a 1.5 T Phillips Achieva scanner (Philips Medical Systems, Best, The
170 Netherlands) as previously described.[13] Each participant was asked not to participate in strenuous
171 exercise or drink alcohol 24 hours before their scan. Each participant was also requested not to eat
172 and only to drink water from 20:00 on the evening before their scan until the scan was completed.
173 Trained research staff measured participants' height and weight from which BMI was calculated in
174 kg/m².

175

176 **Free Living Activity**

177 Immediately after the MRI scan, an ActivPal accelerometer/ inclinometer device
178 (<http://www.paltech.plus.com>) was attached to the participant's thigh mid-way between their right
179 hip and knee. The device was worn all day every day (including during sleep and bathing) for seven
180 full consecutive days. The ActivPal classifies an individual's free living activity into periods spent

181 sitting, standing and walking, which it has been validated for. For a detailed discussion on wear
182 protocol and validation see Smith et al.[14] Bespoke software provided by Paltech was used to
183 categorise activity periods into sitting/lying, standing, and stepping, in addition to providing average
184 daily step count. The data are presented as average daily waking time in hours per day (classified as
185 07:00 to 23:59) spent, sitting, standing, and stepping.

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187 Ethics

188 Written informed consent was obtained from all subjects, and the protocol was approved by the
189 Hammersmith Research Ethics Committee (ref nos: 07/Q0411/19 and 06/Q0411/173).

190

191 Analysis

192 Characteristics of the study population, average daily time spent sitting, standing and walking, and
193 the main body composition outcomes were summarised using descriptive statistics. We performed
194 Pearson Correlations to examine associations between different activity categories and the body
195 composition data. For illustrative purposes we also created a standing: sitting ratio and derived two
196 groups based on the median split (high and low). Independent T-tests were performed to compare
197 the main body composition outcomes between groups of high and low standing: sitting ratio. We
198 extracted the partial R^2 statistic from the correlation between sitting time and liver adiposity to
199 inform a power calculation to provide a sample size for the main trial.

200

201 Results

202 Of the 12 females who took part all provided valid MRI and ActivPal data. The volunteers had a
203 mean age of 30.9 ± 6.1 yrs, a mean BMI of 22.9 ± 3.4 , and achieved an average of 9993 ± 5146 steps
204 a day (Table 1 contains all descriptive statistics for the study sample). On average participants spent
205 12.7 ± 1.3 hours a day sitting, 3.2 ± 0.9 hours a day standing, 1.8 ± 0.8 hours a day stepping and the
206 remainder in sleep.

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Table 1: Descriptive statistics of study sample

Variable	Mean/SD	Range
Age	30.9 ±6.1	24 to 45
BMI (kg/m ²)	22.9 ±3.4	18.1 to 28.1
Total body adipose tissue (L)	24.1 ±9.9	13.2 to 44.4
Liver adiposity (%)	0.52 ±0.73	0.12 to 2.56
Visceral/subcutaneous abdominal fat ratio	0.25 ±0.09	0.13 to 0.38
Average sitting time (hr/d)	12.7 ±1.3	11.0 to 15.0
Average standing time (hr/d)	3.2 ±0.9	1.4 to 4.4
Average stepping (hr/d)	1.8 ±0.8	0.6 to 3.1
Average daily step count	9,993 ±5,146	2,918 to 19,995
Average daily energy expenditure (MET-hr)	24.4 ±2.3	22.6 to 30.2

n=12

There were significant correlations between average daily sitting and liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively); standing: sitting ratio was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.53$ and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively; Table 2). We observed weak associations between all activity categories with BMI. Figure One presents comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes. A higher ratio of standing: sitting was consistently associated with lower levels of adiposity.

Table 2: Correlations between ActivPal and MRI measures

	BMI	Total adiposity	Liver fat	Visceral/subcutaneous abdominal fat ratio
Sitting	-0.09	0.10	0.66*	0.64*
Stand: sit ratio	0.24	0.08	-0.53†	-0.45
Av Step-count	-0.22	-0.46	-0.45	-0.51†

n=12; Data are Pearson correlations (r). * $p<0.05$; † $p<0.10$

228 Power Calculation

229 A power calculation was carried out in G-Power to provide a sample size for the main trial. The
230 calculation was based on the correlation between sitting time and liver fat: per 1hr/d sitting was
231 associated with 0.48 [SE, 0.17] unit increase in liver fat [Partial R²= 0.43]. In G-power this equates to
232 an effect size f²= 0.75, and suggests that a sample size of 20 would provide us with 95% power at
233 5% significance level (two-tailed) to detect differences.

235 DISCUSSION

236 The aim of the present study was to investigate the association between objectively measured
237 sitting and standing, using a postural allocation technique, with MRI assessed body composition.
238 Average daily sitting time was associated with liver adiposity and visceral/ subcutaneous abdominal
239 fat ratio. Previous studies have attempted to investigate these associations, but without the benefit
240 of the existing gold standard techniques for body composition or full postural allocation
241 measurements. In a recent study using computed tomography, self-reported leisure time sitting was
242 associated with pericardial fat, but not with any other fat depots.[16] We have previously reported
243 associations between objectively assessed sedentary time (Actigraph) and pericardial fat although
244 the relationship did not persist after adjusting for MVPA.[16] Numerous studies have been carried
245 out to investigate the relationship between sedentary time and BMI in adults and found mixed
246 results. For example, one study carried out in a sample of 881 adults residing in Australia found no
247 significant relationship between change in participant-reported TV viewing time and change in BMI,
248 although a cross-sectional association was found between TV viewing time and BMI at baseline, in
249 females only.[17] In another study carried out in a sample of 3127 adults residing in Southern
250 France, participant-reported TV viewing time was associated with BMI in both sexes.[18] In the
251 Whitehall II prospective study, BMI predicted sitting time at follow-up but the converse was not
252 found.[19] Conflicting findings may be partially explained by the fact that BMI is a poor indicator of
253 adiposity. Moreover, participants may not be able to recall TV viewing time accurately and TV
254 viewing time may be a poor indicator of total sitting.

255 In comparison to previous research the present study used precise objective measures of both
256 sitting time and body composition. Interestingly, a higher ratio of standing: sitting was associated
257 with lower levels of total, and liver adiposity, and visceral/ subcutaneous fat ratio, providing
258 preliminary cross-sectional evidence of the potential influence of light PA (standing) on body

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composition. These findings, although using a more proximal outcome, support previous literature that has found self-reported standing time is inversely related to CVD mortality, in adults.[20]

The present pilot study found weak associations between all activity categories and BMI. BMI is a poor measure of adiposity in comparison to MRI since it cannot distinguish between visceral and subcutaneous fat depots. Since visceral and ectopic fat are thought to be more detrimental to health than subcutaneous,[10,21] it is important to distinguish between different types of fat. Furthermore, the numerator in the BMI calculation “total body weight” does not distinguish between lean and fat mass. Therefore, an individual with high levels of lean mass may be classified as having a high BMI; whereas an individual who is of normal weight but has excess body fat may be classified as having a normal BMI. This might partly explain why several exercise training studies have demonstrated reductions in visceral adiposity in the absence of weight loss.[11]

The *data collection* protocol and tools used within this small pilot study are feasible and can be implemented into the subsequent experimental trial; a 100% response rate was achieved and no participant dropped out of the study. Moreover, all participants provided a full ActivPal dataset (seven complete days) and adhered to the wear protocol. However, it should be noted that the subsequent experimental trial will require two identical data collection sessions to assess the impact of displacing sitting with standing on body composition.

It was not feasible to make multiple statistical adjustments in our analyses owing to the small sample size, thus associations between sitting and adiposity may have been confounded by vigorous exercise. However, we selected a homogenous sample and simple correlations suggested far weaker associations between average step count and adiposity. Given the cross-sectional nature of this pilot study the direction of the observed associations remains unknown. Moreover, the representativeness of the findings are limited, owing to the small sample size of healthy Caucasian females residing in London. However, the aim of this pilot study was to provide novel data to support the underlying rationale and generate a sample size for a subsequent experimental trial.

CONCLUSION

This pilot study has provided preliminary evidence of the strong relationships between objectively measured sitting and standing and precise measures of body composition.

290 **Competing interests**

291 The authors declare that they have no competing interests.

292

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296 study design; in the collection, analysis and interpretation of data; in writing of the report; or in the
297 decision to submit the paper for publication.

298

299 **Authors' contributions**

300 LS and MH designed the study. All authors contributed to development of the study protocol. LS and
301 MH drafted the manuscript. LT and JB assisted in drafting the manuscript. All authors read and
302 approved the final manuscript.

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Physical activity, sedentary behaviour, adiposity, magnetic resonance imaging

ABSTRACT

Objective: To investigate the association between objectively measured sitting and standing, using a postural allocation technique, with MRI assessed body composition.

Design: The present study was a cross-sectional pilot study.

Setting: Participants were examined at one centre located in London, UK.

Participants: Normal weight Caucasian females (30.9 ± 6.1 yrs; BMI, 22.9 ± 3.4 kg/m²) with desk bound occupations were recruited to minimise variability in body composition outcomes. A convenience sample of 12 females were recruited in January 2014 from University College London

Outcome measures: For each participant a number of body composition variables were attained from a single whole-body magnetic resonance imaging session. Main outcome variables included: total and liver adiposity, visceral/subcutaneous fat ratio, and body mass index. Main exposure variables included: average sitting time, standing: sitting ratio, and step count. Pearson Correlations were carried out to examine associations between different activity categories and body composition variables.

Results: There were significant correlations between average daily sitting and liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively); standing: sitting ratio was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.53$ and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively).

Conclusion: This pilot study has provided preliminary evidence of relationships between objectively measured sitting and standing and precise measures of body composition.

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62 **Article Summary: strengths and limitations of this study**

- 63 • This is the first study to show an association between objectively measured sitting and
64 standing, using a postural allocation technique, with MRI assessed body composition.
- 65 • The data collection protocol and tools used within this pilot study are feasible and can be
66 implemented into subsequent experimental trials.
- 67 • It was not feasible to make multiple statistical adjustments in our analyses owing to the
68 small sample size.

For peer review only

INTRODUCTION

There is a growing body of literature that suggests sedentary behaviours – defined as any waking behaviour characterised by energy expenditure below 1.5 metabolic equivalents while in a sitting or reclined posture – are associated with higher risk of cardiovascular disease (CVD) and mortality, after statistical adjustment for moderate-to-vigorous intensity physical activities (MVPA; e.g. brisk walking).[1] This has large public health relevance in light of objective data from general adult population studies in the USA and Great Britain that show on average adults spend approximately 60 to 70% of their waking hours in sedentary behaviours.[2] Indeed, westernised society is geared towards promoting sedentary lifestyles (i.e. screen based entertainment, motorised transport etc.), thus, developing strategies to combat sedentary behaviour are crucial.

Such a strategy might not necessarily involve exercise of moderate or vigorous intensity, as interventions to increase exercise levels have proved challenging.[3, 4] Instead, given the barriers to structured exercise (e.g. motivation, cost, access and time etc.), we might consider more subtle lifestyle approaches that are primarily designed to displace sedentary behaviour (i.e. sitting) with forms of lighter intensity (incidental) activity (e.g. standing). If lifestyle population activity patterns can be shifted from predominantly sedentary to the next lowest physical activity (PA) category (standing), this may have public health benefit given the low proportion of individuals meeting current PA guidelines.

To date, limited epidemiological evidence has been generated on the associations between light intensity activity and health. This is partially owing to measurement issues; self-reported PA questionnaires are designed to capture MVPA and there are technical limitations in differentiating between sitting, standing and other forms of light activity when interpreting objective activity data. The most commonly used accelerometer, the Actigraph, quantifies time spent in different intensities of activity by summing time above and below specified count thresholds. This method works reasonably well for identifying MVPA, but is less accurate for distinguishing *between* sedentary and light activity (i.e. between sitting and standing).[5] Thus, methods that employ postural allocation may be more reliable, which have only recently become readily available.

Some experimental evidence is beginning to emerge in this area. For example, one study manipulated sitting time and PA over one day under free living conditions. The results indicated that replacing sitting with longer periods of light activity was more beneficial for metabolic health than one hour of vigorous exercise despite equivalent daily energy expenditure in each treatment group.[6] In a laboratory controlled trial conducted over an 8 hour period, interrupting sitting time

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every 20 mins with short 2-min bouts of light- or moderate intensity walking was shown to lower postprandial glucose and insulin levels in overweight/ obese adults.[7] In another study, using continuously monitored capillary blood glucose, there was a 43% reduction in blood glucose excursion during an afternoon (185 minutes) of standing compared with sitting in desk-based workers.[8] In a pilot study replacing sitting workstations with sit-stand workstations employees reduced sitting time by 137 min/d and increases in HDL-cholesterol were observed at 3 months follow-up.[9] However, the biological mechanisms underlying these effects still remain unclear, although increased muscle activation during standing could be an important underlying mechanism, for example, by increasing skeletal muscle metabolism. Replacing a sitting workstation with a standing workstation was shown to increase daily energy expenditure,[8] thus the longer term benefits might also include reductions in total, visceral, and liver adiposity. A reduction in total and visceral adiposity is known to have a favourable impact on a range of CVD risk factors including inflammatory markers, lipids, and glycaemic control.[10] Liver adiposity is of particular interest as it has been linked to metabolic risk and worsening insulin resistance.[11] Several exercise training studies have demonstrated reductions in visceral adiposity in the absence of weight loss.[12] However, the relationship between light PA (standing) and total, visceral, and liver adiposity has yet to be investigated using precise imaging techniques.

Further research is needed to aid in the understanding of the relationships between objectively measuring sitting and standing, using an objective postural allocation technique, and measures of total, visceral and liver adiposity, using precise imaging techniques. This will contribute to the small but growing body of literature that aims to inform policy and intervention on the health benefits of displacing sitting with standing.

AIM

In the present study, several contemporary methods were used, including an objective postural allocation technique in combination with magnetic resonance imaging (MRI), to aid in the understanding of the relationships between sitting/ standing and body composition. This data collection was primarily designed to inform a large experimental trial that will investigate the impact of displacing sitting with standing on total, visceral, and liver adiposity. This pilot data will (i) inform the underlying rationale of the trial by producing evidence, if it exists, of relationships between objective measures of sitting and standing and body composition, and (ii) generate an effect size on which to base sample size calculations to inform the main trial.

METHODS

Design, participants and sample size

This cross-sectional pilot study was carried out in 12 healthy Caucasian females. The sample size for this pilot was based on previous published work, which has shown that significant differences in body composition could be readily observed in cross-sectional studies of 10 or less volunteers.[13] Normal weight females with desk bound occupations were selected from a larger cohort to minimise variability in age, weight and overall anthropometry. A convenience sample was recruited in January 2014 from University College London. We randomly invited 12 females who met our criteria to take part in the study. All females invited agreed to take part. One week prior to data collection trained research staff met with the participants to administer the participant information sheets and explain the study protocol.

Measures of adiposity

For each participant, a range of body composition variables were attained from a single whole-body MRI session lasting approximately 45 mins. For the purpose of the present study we defined our main outcomes as *a priori*, which included body mass index (BMI), total litres of body adiposity (L), liver adiposity (%), and visceral/subcutaneous abdominal fat ratio. Whole-body MR images and liver adiposity (%) were obtained on a 1.5 T Phillips Achieva scanner (Philips Medical Systems, Best, The Netherlands) as previously described.[14] Each participant was asked not to participate in strenuous exercise or drink alcohol 24 hours before their scan. Each participant was also requested not to eat and only to drink water from 20:00 on the evening before their scan until the scan was completed. Trained research staff measured participants' height and weight from which BMI was calculated in kg/m².

Free Living Activity

Immediately after the MRI scan, an ActivPal accelerometer/ inclinometer device (<http://www.paltechnologies.com/>) was attached to the participant's thigh mid-way between their right hip and knee. The ActivPal classifies an individual's free living activity into periods spent sitting, standing and walking, which it has been validated for [15]. The ActivPals inclinometer and unique

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positioning on the thigh allows the device to distinguish between sitting and standing using proprietary algorithms, which previous objective physical activity monitors have been unable to do. The device was worn all day every day (including during sleep and bathing) for seven full consecutive days. Bespoke software provided by Paltech was used to categorise activity periods into sitting/lying, standing, and stepping, in addition to providing average daily step count. The data are presented as average daily waking time in hours per day (classified as 07:00 to 23:59) spent, sitting, standing, and stepping.

Ethics

Written informed consent was obtained from all subjects, and the protocol was approved by the Hammersmith Research Ethics Committee (ref nos: 07/Q0411/19 and 06/Q0411/173).

Analysis

Characteristics of the study population, average daily time spent sitting, standing and walking, and the main body composition outcomes were summarised using descriptive statistics. We performed Pearson Correlations to examine associations between different activity categories and the body composition data. For illustrative purposes we also created a standing: sitting ratio and derived two groups based on the median split (high and low). Independent T-tests were performed to compare the main body composition outcomes between groups of high and low standing: sitting ratio. We extracted the partial R² statistic from the correlation between sitting time and liver adiposity to inform a power calculation to provide a sample size for the main trial.

Results

Of the 12 females who took part all provided valid MRI and ActivPal data. The volunteers had a mean age of 30.9 ± 6.1 yrs, a mean BMI of 22.9 ± 3.4, and achieved an average of 9993 ± 5146 steps a day (Table 1 contains all descriptive statistics for the study sample). On average participants spent 12.7 ± 1.3 hours a day sitting, 3.2 ± 0.9 hours a day standing, 1.8 ± 0.8 hours a day stepping and the remainder in sleep.

Table 1: Descriptive statistics of study sample

Variable	Mean/SD	Range
Age	30.9 ±6.1	24 to 45
BMI (kg/m ²)	22.9 ±3.4	18.1 to 28.1
Total body adipose tissue (L)	24.1 ±9.9	13.2 to 44.4
Liver adiposity (%)	0.52 ±0.73	0.12 to 2.56
Visceral/subcutaneous abdominal fat ratio	0.25 ±0.09	0.13 to 0.38
Average sitting time (hr/d)	12.7 ±1.3	11.0 to 15.0
Average standing time (hr/d)	3.2 ±0.9	1.4 to 4.4
Average stepping (hr/d)	1.8 ±0.8	0.6 to 3.1
Average daily step count	9,993 ±5,146	2,918 to 19,995
Average daily energy expenditure (MET-hr)	24.4 ±2.3	22.6 to 30.2

n=12

There were significant correlations between average daily sitting and liver adiposity and visceral/subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively); standing: sitting ratio was moderately correlated with liver adiposity and visceral/subcutaneous abdominal fat ratio ($r=-0.53$ and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total adiposity and visceral/subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively; Table 2). Scatter plots of these associations are presented as supplementary material (see supplementary data; Figures S1-4). We observed weak associations between all activity categories with BMI. Figure One presents comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes. A higher ratio of standing: sitting was consistently associated with lower levels of adiposity. Since the observed associations with sitting may have been influenced by vigorous exercise, we performed sensitivity analyses that removed two participants recording over 15,000 steps (indicative of vigorous exercise). In these analyses ($n=10$) the associations between sitting and adiposity remained largely unchanged; average daily sitting remained associated with liver adiposity ($r=0.65$, $p=0.043$) and visceral/subcutaneous abdominal fat ratio ($r=0.73$, $p=0.017$).

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Table 2: Correlations between ActivPal and MRI measures

	BMI	Total adiposity	Liver fat	Visceral/subcutaneous abdominal fat ratio
Sitting	-0.09	0.10	0.66*	0.64*
Stand: sit ratio	0.24	0.08	-0.53†	-0.45
Av Step-count	-0.22	-0.46	-0.45	-0.51†

n=12; Data are Pearson correlations (r). * $p<0.05$; † $p<0.1$

238 Power Calculation

239 A power calculation was carried out in G-Power to provide a sample size for the main trial. The
240 calculation was based on the correlation between sitting time and liver fat: per 1hr/d sitting was
241 associated with 0.48 [SE, 0.17] unit increase in liver fat [Partial R²= 0.43]. In G-power this equates to
242 an effect size f²= 0.75, and suggests that a sample size of 20 per group would provide us with 95%
243 power at 5% significance level (two-tailed) to detect differences.

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245 DISCUSSION

246 The aim of the present study was to investigate the association between objectively measured
247 sitting and standing, using a postural allocation technique (an accelerometer/ inclinometer attached
248 to the participant's thigh mid-way between the hip and the knee), with MRI assessed body
249 composition. Average daily sitting time was associated with liver adiposity and visceral/
250 subcutaneous abdominal fat ratio. Previous studies have attempted to investigate these
251 associations, but without the benefit of the existing gold standard techniques for body composition
252 or full postural allocation measurements. In a recent study using computed tomography, self-
253 reported leisure time sitting was associated with pericardial fat, but not with any other fat
254 depots.[16] We have previously reported associations between objectively assessed sedentary time
255 (Actigraph) and pericardial fat although the relationship did not persist after adjusting for MVPA.[17]
256 Numerous studies have been carried out to investigate the relationship between sedentary time and
257 BMI in adults and found mixed results. For example, one study carried out in a sample of 881 adults
258 residing in Australia found no significant relationship between change in participant-reported TV
259 viewing time and change in BMI, although a cross-sectional association was found between TV
260 viewing time and BMI at baseline, in females only.[18] In another study carried out in a sample of
261 3127 adults residing in Southern France, participant-reported TV viewing time was associated with
262 BMI in both sexes.[19] In the Whitehall II prospective study, BMI predicted sitting time at follow-up
263 but the converse was not found.[20] Conflicting findings may be partially explained by the fact that
264 BMI is a poor indicator of adiposity. Moreover, participants may not be able to recall TV viewing
265 time accurately and TV viewing time may be a poor indicator of total sitting.

266 In comparison to previous research the present study used precise objective measures of both
267 sitting time and body composition. Interestingly, a higher ratio of standing: sitting was associated
268 with lower levels of total, and liver adiposity, and visceral/ subcutaneous fat ratio, providing
269 preliminary cross-sectional evidence of the potential influence of light PA (standing) on body

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composition. These findings, although using a more proximal outcome, support previous literature that has found self-reported standing time is inversely related to CVD mortality, in adults.[21]

The present pilot study found weak associations between all activity categories and BMI. BMI is a poor measure of adiposity in comparison to MRI since it cannot distinguish between visceral and subcutaneous fat depots. Since visceral and ectopic fat are thought to be more detrimental to health than subcutaneous,[10,11] it is important to distinguish between different types of fat. Furthermore, the numerator in the BMI calculation “total body weight” does not distinguish between lean and fat mass. Therefore, an individual with high levels of lean mass may be classified as having a high BMI; whereas an individual who is of normal weight but has excess body fat may be classified as having a normal BMI. This might partly explain why several exercise training studies have demonstrated reductions in visceral adiposity in the absence of weight loss.[12]

The *data collection* protocol and tools used within this small pilot study are feasible and can be implemented into the subsequent experimental trial; a 100% response rate was achieved and no participant dropped out of the study. Moreover, all participants provided a full ActivPal dataset (seven complete days) and adhered to the wear protocol. However, it should be noted that the subsequent experimental trial will require two identical data collection sessions to assess the impact of displacing sitting with standing on body composition.

It was not feasible to make multiple statistical adjustments in our analyses owing to the small sample size, thus associations between sitting and adiposity may have been confounded by vigorous exercise. However, we selected a homogenous sample and the removal of two highly active participants in our sample did not change the results. Given the cross-sectional nature of this pilot study the direction of the observed associations remains unknown. Moreover, the representativeness of the findings are limited, owing to the small sample size of healthy Caucasian females residing in London. However, the aim of this pilot study was to provide novel data to support the underlying rationale and generate a sample size for a subsequent experimental trial.

Inclusion criteria for the experimental trial, that this pilot study was carried out to inform, will be overweight/ obesity. We will use a number of biomedical outcomes in the main trial including body composition (MRI), and biochemical risk markers (lipids, inflammatory markers, glucose).

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3 301 **CONCLUSION**
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5 302 This pilot study has provided preliminary evidence of the strong relationships between objectively
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7 303 measured sitting and standing (an accelerometer/ inclinometer attached to the participant's thigh
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9 304 mid-way between the hip and the knee) and precise measures of body composition.
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Authors’ contributions

LS and MH designed the study. All authors contributed to development of the study protocol. LS and MH drafted the manuscript. LT and JB assisted in drafting the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Data Sharing Statement

No additional data is available.

Figure Legend

Figure 1 Comparison between groups of high and low standing: sitting ratio in relation to the main body composition outcomes.

Supplementary Figures:

- Supplementary Figure 1. Scatter plot of sitting time against body mass index.
- Supplementary Figure 2. Scatter plot of sitting time against total adiposity.
- Supplementary Figure 3. Scatter plot of sitting time against liver fat.
- Supplementary Figure 4. Scatter plot of sitting time against Visceral/subcutaneous abdominal fat ratio.

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The association between objectively measured sitting and standing with body composition: A pilot study using magnetic resonance imaging

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30 **ABSTRACT**

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32 **Objective:** To investigate the association between objectively measured sitting and standing, using a
33 postural allocation technique, with MRI assessed body composition.

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35 **Design:** The present study was a cross-sectional pilot study.

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37 **Setting:** Participants were examined at one centre located in London, UK.

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39 **Participants:** Normal weight Caucasian females (30.9 ± 6.1 yrs; BMI, 22.9 ± 3.4 kg/m²) with desk
40 bound occupations were recruited to minimise variability in body composition outcomes. A
41 convenience sample of 12 females were recruited in January 2014 from University College London

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43 **Outcome measures:** For each participant a number of body composition variables were attained
44 from a single whole-body magnetic resonance imaging session. Main outcome variables included:
45 total and liver adiposity, visceral/subcutaneous fat ratio, and body mass index. Main exposure
46 variables included: average sitting time, standing: sitting ratio, and step count. Pearson Correlations
47 were carried out to examine associations between different activity categories and body
48 composition variables.

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50 **Results:** There were significant correlations between average daily sitting and liver adiposity and
51 visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively); standing: sitting ratio
52 was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-$
53 0.53 and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total
54 adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively).

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56 **Conclusion:** This pilot study has provided preliminary evidence of relationships between objectively
57 measured sitting and standing and precise measures of body composition.

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Article Summary: strengths and limitations of this study

- This is the first study to show an association between objectively measured sitting and standing, using a postural allocation technique, with MRI assessed body composition.
- The data collection protocol and tools used within this pilot study are feasible and can be implemented into subsequent experimental trials.
- It was not feasible to make multiple statistical adjustments in our analyses owing to the small sample size.

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87 INTRODUCTION

88 There is a growing body of literature that suggests sedentary behaviours – defined as any waking
89 behaviour characterised by energy expenditure below 1.5 metabolic equivalents while in a sitting or
90 reclined posture – are associated with higher risk of cardiovascular disease (CVD) and mortality, after
91 statistical adjustment for moderate-to-vigorous intensity physical activities (MVPA; e.g. brisk
92 walking).[1] This has large public health relevance in light of objective data from general adult
93 population studies in the USA and Great Britain that show on average adults spend approximately 60
94 to 70% of their waking hours in sedentary behaviours.[2] Indeed, westernised society is geared
95 towards promoting sedentary lifestyles (i.e. screen based entertainment, motorised transport etc.),
96 thus, developing strategies to combat sedentary behaviour are crucial.

97 Such a strategy might not necessarily involve exercise of moderate or vigorous intensity, as
98 interventions to increase exercise levels have proved challenging.[3, 4] Instead, given the barriers to
99 structured exercise (e.g. motivation, cost, access and time etc.), we might consider more subtle
100 lifestyle approaches that are primarily designed to displace sedentary behaviour (i.e. sitting) with
101 forms of lighter intensity (incidental) activity (e.g. standing). If lifestyle population activity patterns
102 can be shifted from predominantly sedentary to the next lowest physical activity (PA) category
103 (standing), this may have public health benefit given the low proportion of individuals meeting
104 current PA guidelines.

105 To date, limited epidemiological evidence has been generated on the associations between light
106 intensity activity and health. This is partially owing to measurement issues; self-reported PA
107 questionnaires are designed to capture MVPA and there are technical limitations in differentiating
108 between sitting, standing and other forms of light activity when interpreting objective activity data.
109 The most commonly used accelerometer, the Actigraph, quantifies time spent in different intensities
110 of activity by summing time above and below specified count thresholds. This method works
111 reasonably well for identifying MVPA, but is less accurate for distinguishing between sedentary and
112 light activity (i.e. between sitting and standing).[5] Thus, methods that employ postural allocation
113 may be more reliable, which have only recently become readily available.

114 Some experimental evidence is beginning to emerge in this area. For example, one study
115 manipulated sitting time and PA over one day under free living conditions. The results indicated that
116 replacing sitting with longer periods of light activity was more beneficial for metabolic health than
117 one hour of vigorous exercise despite equivalent daily energy expenditure in each treatment
118 group.[6] In a laboratory controlled trial conducted over an 8 hour period, interrupting sitting time

every 20 mins with short 2-min bouts of light- or moderate intensity walking was shown to lower postprandial glucose and insulin levels in overweight/ obese adults.[7] In another study, using continuously monitored capillary blood glucose, there was a 43% reduction in blood glucose excursion during an afternoon (185 minutes) of standing compared with sitting in desk-based workers.[8] In a pilot study replacing sitting workstations with sit-stand workstations employees reduced sitting time by 137 min/d and increases in HDL-cholesterol were observed at 3 months follow-up.[9] However, the biological mechanisms underlying these effects still remain unclear, although increased muscle activation during standing could be an important underlying mechanism, for example, by increasing skeletal muscle metabolism. Replacing a sitting workstation with a standing workstation was shown to increase daily energy expenditure,[8] thus the longer term benefits might also include reductions in total, visceral, and liver adiposity. A reduction in total and visceral adiposity is known to have a favourable impact on a range of CVD risk factors including inflammatory markers, lipids, and glycaemic control.[10] Liver adiposity is of particular interest as it has been linked to metabolic risk and worsening insulin resistance.[11] Several exercise training studies have demonstrated reductions in visceral adiposity in the absence of weight loss.[12] However, the relationship between light PA (standing) and total, visceral, and liver adiposity has yet to be investigated using precise imaging techniques.

Further research is needed to aid in the understanding of the relationships between objectively measuring sitting and standing, using an objective postural allocation technique, and measures of total, visceral and liver adiposity, using precise imaging techniques. This will contribute to the small but growing body of literature that aims to inform policy and intervention on the health benefits of displacing sitting with standing.

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142 AIM

143 In the present study, several contemporary methods were used, including an objective postural allocation technique in combination with magnetic resonance imaging (MRI), to aid in the understanding of the relationships between sitting/ standing and body composition. This data collection was primarily designed to inform a large experimental trial that will investigate the impact of displacing sitting with standing on total, visceral, and liver adiposity. This pilot data will (i) inform the underlying rationale of the trial by producing evidence, if it exists, of relationships between objective measures of sitting and standing and body composition, and (ii) generate an effect size on which to base sample size calculations to inform the main trial.

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METHODS

Design, participants and sample size

This cross-sectional pilot study was carried out in 12 healthy Caucasian females. The sample size for this pilot was based on previous published work, which has shown that significant differences in body composition could be readily observed in cross-sectional studies of 10 or less volunteers.[13] Normal weight females with desk bound occupations were selected from a larger cohort to minimise variability in age, weight and overall anthropometry. A convenience sample was recruited in January 2014 from University College London. We randomly invited 12 females who met our criteria to take part in the study. All females invited agreed to take part. One week prior to data collection trained research staff met with the participants to administer the participant information sheets and explain the study protocol.

Measures of adiposity

For each participant, a range of body composition variables were attained from a single whole-body MRI session lasting approximately 45 mins. For the purpose of the present study we defined our main outcomes as *a priori*, which included body mass index (BMI), total litres of body adiposity (L), liver adiposity (%), and visceral/subcutaneous abdominal fat ratio. Whole-body MR images and liver adiposity (%) were obtained on a 1.5 T Phillips Achieva scanner (Philips Medical Systems, Best, The Netherlands) as previously described.[14] Each participant was asked not to participate in strenuous exercise or drink alcohol 24 hours before their scan. Each participant was also requested not to eat and only to drink water from 20:00 on the evening before their scan until the scan was completed. Trained research staff measured participants' height and weight from which BMI was calculated in kg/m².

Free Living Activity

Immediately after the MRI scan, an ActivPal accelerometer/ inclinometer device (<http://www.paltechnologies.com/>) was attached to the participant's thigh mid-way between their right hip and knee. The ActivPal classifies an individual's free living activity into periods spent sitting, standing and walking, which it has been validated for [15]. The ActivPals inclinometer and unique

positioning on the thigh allows the device to distinguish between sitting and standing using proprietary algorithms, which previous objective physical activity monitors have been unable to do.

The device was worn all day every day (including during sleep and bathing) for seven full consecutive days. Bespoke software provided by Paltech was used to categorise activity periods into sitting/lying, standing, and stepping, in addition to providing average daily step count. The data are presented as average daily waking time in hours per day (classified as 07:00 to 23:59) spent, sitting, standing, and stepping.

Ethics

Written informed consent was obtained from all subjects, and the protocol was approved by the Hammersmith Research Ethics Committee (ref nos: 07/Q0411/19 and 06/Q0411/173).

Analysis

Characteristics of the study population, average daily time spent sitting, standing and walking, and the main body composition outcomes were summarised using descriptive statistics. We performed Pearson Correlations to examine associations between different activity categories and the body composition data. For illustrative purposes we also created a standing: sitting ratio and derived two groups based on the median split (high and low). Independent T-tests were performed to compare the main body composition outcomes between groups of high and low standing: sitting ratio. We extracted the partial R^2 statistic from the correlation between sitting time and liver adiposity to inform a power calculation to provide a sample size for the main trial.

Results

Of the 12 females who took part all provided valid MRI and ActivPal data. The volunteers had a mean age of 30.9 ± 6.1 yrs, a mean BMI of 22.9 ± 3.4 , and achieved an average of 9993 ± 5146 steps a day (Table 1 contains all descriptive statistics for the study sample). On average participants spent 12.7 ± 1.3 hours a day sitting, 3.2 ± 0.9 hours a day standing, 1.8 ± 0.8 hours a day stepping and the remainder in sleep.

Table 1: Descriptive statistics of study sample

Variable	Mean/SD	Range
Age	30.9 ±6.1	24 to 45
BMI (kg/m ²)	22.9 ±3.4	18.1 to 28.1
Total body adipose tissue (L)	24.1 ±9.9	13.2 to 44.4
Liver adiposity (%)	0.52 ±0.73	0.12 to 2.56
Visceral/subcutaneous abdominal fat ratio	0.25 ±0.09	0.13 to 0.38
Average sitting time (hr/d)	12.7 ±1.3	11.0 to 15.0
Average standing time (hr/d)	3.2 ±0.9	1.4 to 4.4
Average stepping (hr/d)	1.8 ±0.8	0.6 to 3.1
Average daily step count	9,993 ±5,146	2,918 to 19,995
Average daily energy expenditure (MET-hr)	24.4 ±2.3	22.6 to 30.2

n=12

There were significant correlations between average daily sitting and liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively; see supplementary file one for scatter plots between sitting against body composition outcomes); standing: sitting ratio was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.53$ and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively; Table 2). Scatter plots of these associations are presented as supplementary material (see supplementary data; Figures S1-4). We observed weak associations between all activity categories with BMI. Figure One presents comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes. A higher ratio of standing: sitting was consistently associated with lower levels of adiposity. Since the observed associations with sitting may have been influenced by vigorous exercise, we performed sensitivity analyses that removed two participants recording over 15,000 steps (indicative of vigorous exercise). In these analyses ($n=10$) the associations between sitting and adiposity remained largely unchanged; average daily sitting remained associated with liver adiposity ($r=0.65$, $p=0.043$) and visceral/ subcutaneous abdominal fat ratio ($r=0.73$, $p=0.017$).

Table 2: Correlations between ActivPal and MRI measures

	BMI	Total adiposity	Liver fat	Visceral/subcutaneous abdominal fat ratio
Sitting	-0.09	0.10	0.66*	0.64*
Stand: sit ratio	0.24	0.08	-0.53†	-0.45
Av Step-count	-0.22	-0.46	-0.45	-0.51†

n=12; Data are Pearson correlations (r). * $p<0.05$; † $p<0.1$

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Power Calculation

A power calculation was carried out in G-Power to provide a sample size for the main trial. The calculation was based on the correlation between sitting time and liver fat: per 1hr/d sitting was associated with 0.48 [SE, 0.17] unit increase in liver fat [Partial R²= 0.43]. In G-power this equates to an effect size f²= 0.75, and suggests that a sample size of 20 per group would provide us with 95% power at 5% significance level (two-tailed) to detect differences.

DISCUSSION

The aim of the present study was to investigate the association between objectively measured sitting and standing, using a postural allocation technique (an accelerometer/ inclinometer attached to the participant's thigh mid-way between the hip and the knee), with MRI assessed body composition. Average daily sitting time was associated with liver adiposity and visceral/ subcutaneous abdominal fat ratio. Previous studies have attempted to investigate these associations, but without the benefit of the existing gold standard techniques for body composition or full postural allocation measurements. In a recent study using computed tomography, self-reported leisure time sitting was associated with pericardial fat, but not with any other fat depots.[16] We have previously reported associations between objectively assessed sedentary time (Actigraph) and pericardial fat although the relationship did not persist after adjusting for MVPA.[17] Numerous studies have been carried out to investigate the relationship between sedentary time and BMI in adults and found mixed results. For example, one study carried out in a sample of 881 adults residing in Australia found no significant relationship between change in participant-reported TV viewing time and change in BMI, although a cross-sectional association was found between TV viewing time and BMI at baseline, in females only.[18] In another study carried out in a sample of 3127 adults residing in Southern France, participant-reported TV viewing time was associated with BMI in both sexes.[19] In the Whitehall II prospective study, BMI predicted sitting time at follow-up but the converse was not found.[20] Conflicting findings may be partially explained by the fact that BMI is a poor indicator of adiposity. Moreover, participants may not be able to recall TV viewing time accurately and TV viewing time may be a poor indicator of total sitting.

In comparison to previous research the present study used precise objective measures of both sitting time and body composition. Interestingly, a higher ratio of standing: sitting was associated with lower levels of total, and liver adiposity, and visceral/ subcutaneous fat ratio, providing preliminary cross-sectional evidence of the potential influence of light PA (standing) on body

271 composition. These findings, although using a more proximal outcome, support previous literature
272 that has found self-reported standing time is inversely related to CVD mortality, in adults.[21]

273 The present pilot study found weak associations between all activity categories and BMI. BMI is a
274 poor measure of adiposity in comparison to MRI since it cannot distinguish between visceral and
275 subcutaneous fat depots. Since visceral and ectopic fat are thought to be more detrimental to health
276 than subcutaneous,[10,11] it is important to distinguish between different types of fat. Furthermore,
277 the numerator in the BMI calculation “total body weight” does not distinguish between lean and fat
278 mass. Therefore, an individual with high levels of lean mass may be classified as having a high BMI;
279 whereas an individual who is of normal weight but has excess body fat may be classified as having a
280 normal BMI. This might partly explain why several exercise training studies have demonstrated
281 reductions in visceral adiposity in the absence of weight loss.[12]

282 The *data collection* protocol and tools used within this small pilot study are feasible and can be
283 implemented into the subsequent experimental trial; a 100% response rate was achieved and no
284 participant dropped out of the study. Moreover, all participants provided a full ActivPal dataset
285 (seven complete days) and adhered to the wear protocol. However, it should be noted that the
286 subsequent experimental trial will require two identical data collection sessions to assess the impact
287 of displacing sitting with standing on body composition.

288 It was not feasible to make multiple statistical adjustments in our analyses owing to the small
289 sample size, thus associations between sitting and adiposity may have been confounded by vigorous
290 exercise. However, we selected a homogenous sample and the removal of two highly active
291 participants in our sample did not change the results. Given the cross-sectional nature of this pilot
292 study the direction of the observed associations remains unknown. Moreover, the
293 representativeness of the findings are limited, owing to the small sample size of healthy Caucasian
294 females residing in London. However, the aim of this pilot study was to provide novel data to
295 support the underlying rationale and generate a sample size for a subsequent experimental trial.

296 Inclusion criteria for the experimental trial, that this pilot study was carried out to inform, will be
297 overweight/ obesity. We will use a number of biomedical outcomes in the main trial including body
298 composition (MRI), and biochemical risk markers (lipids, inflammatory markers, glucose).

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CONCLUSION

This pilot study has provided preliminary evidence of the strong relationships between objectively measured sitting and standing (an accelerometer/ inclinometer attached to the participant’s thigh mid-way between the hip and the knee) and precise measures of body composition.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

LS and MH designed the study. All authors contributed to development of the study protocol. LS and MH drafted the manuscript. LT and JB assisted in drafting the manuscript. All authors read and approved the final manuscript.

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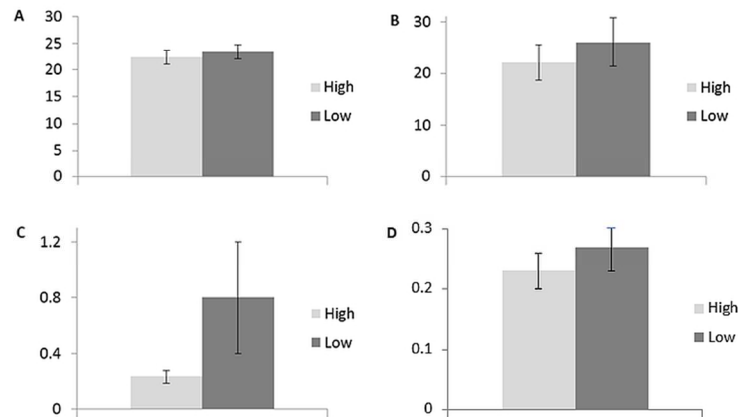
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Figure 1: comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes



A, Body mass index; B, Total adipose fat (litres); C, liver fat (%); D, visceral/subcutaneous abdominal fat ratio; n=12

The high: low cut point was ≥ 0.27

90x67mm (300 x 300 DPI)

Supplementary data

Figure 1. Scatter plot of sitting time against body mass index.

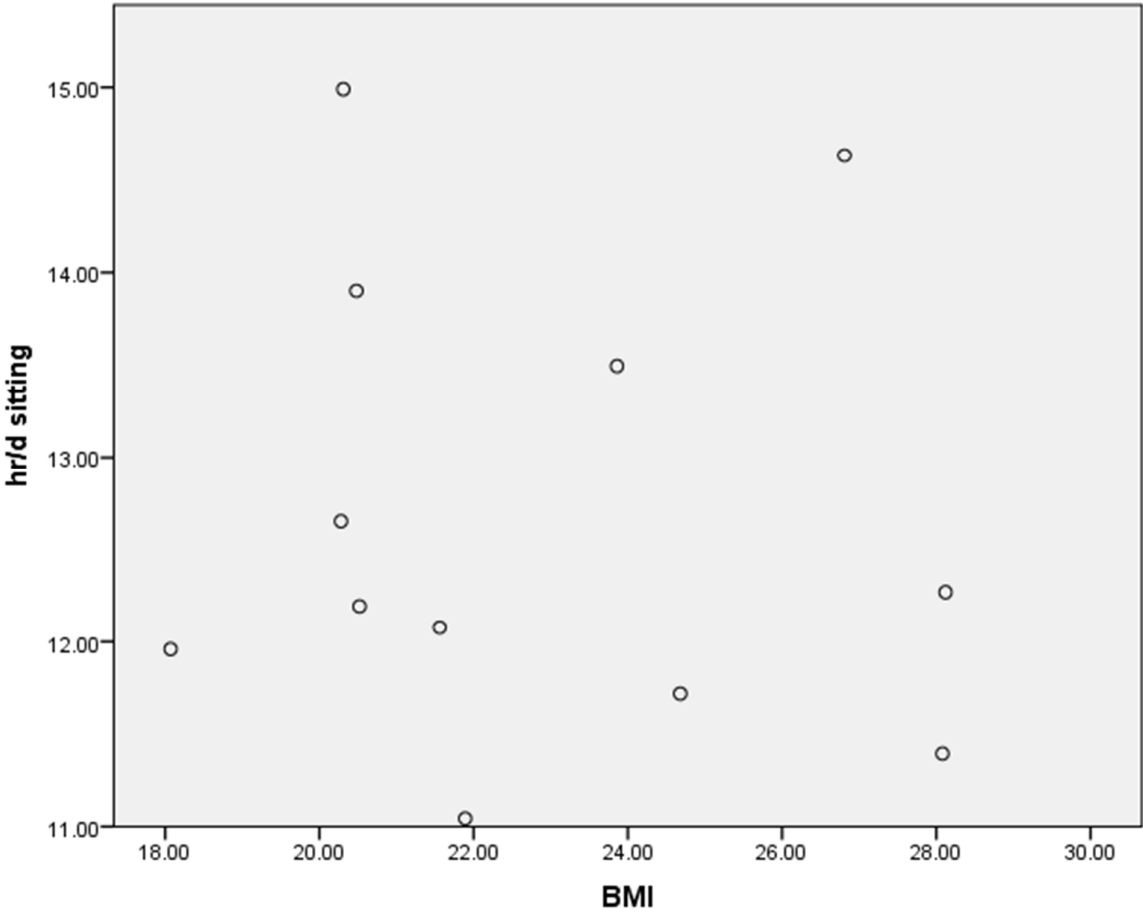


Figure 2. Scatter plot of sitting time against total adiposity.

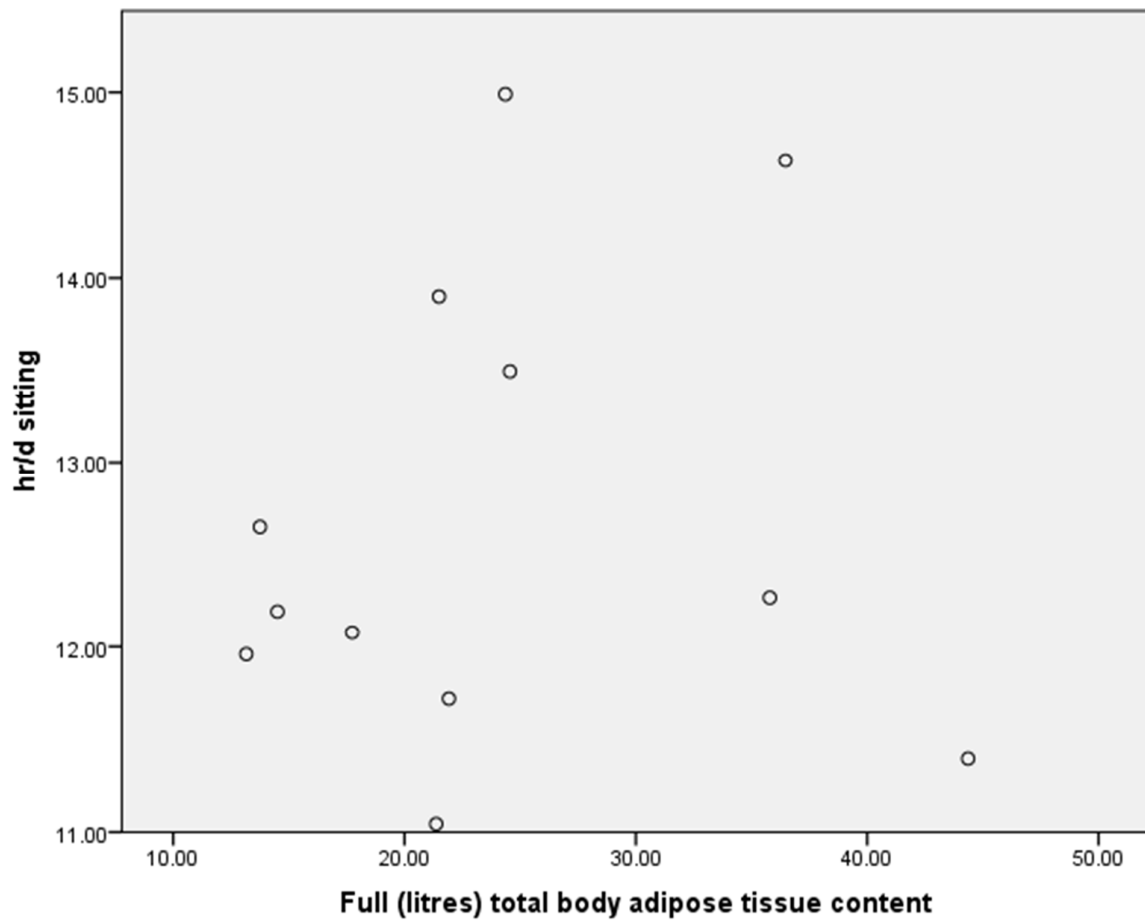


Figure 3. Scatter plot of sitting time against liver fat.

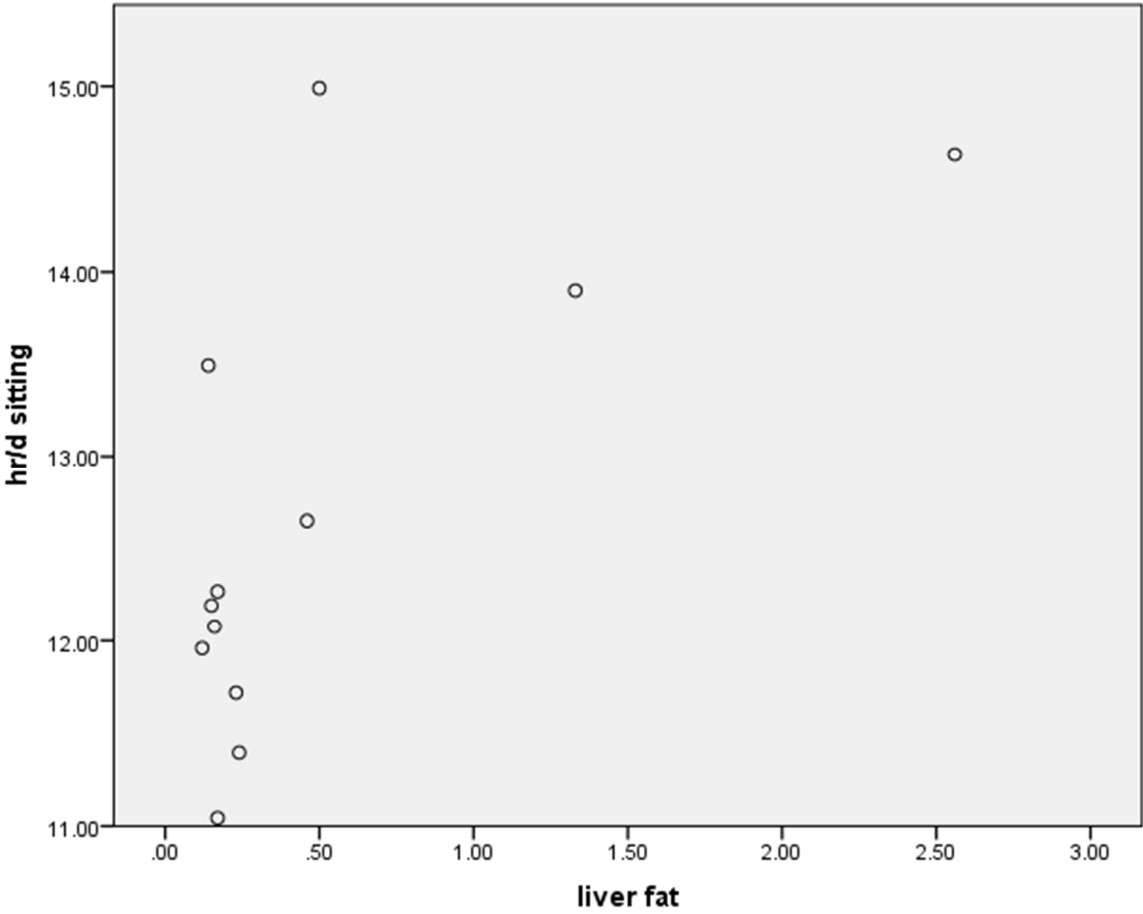
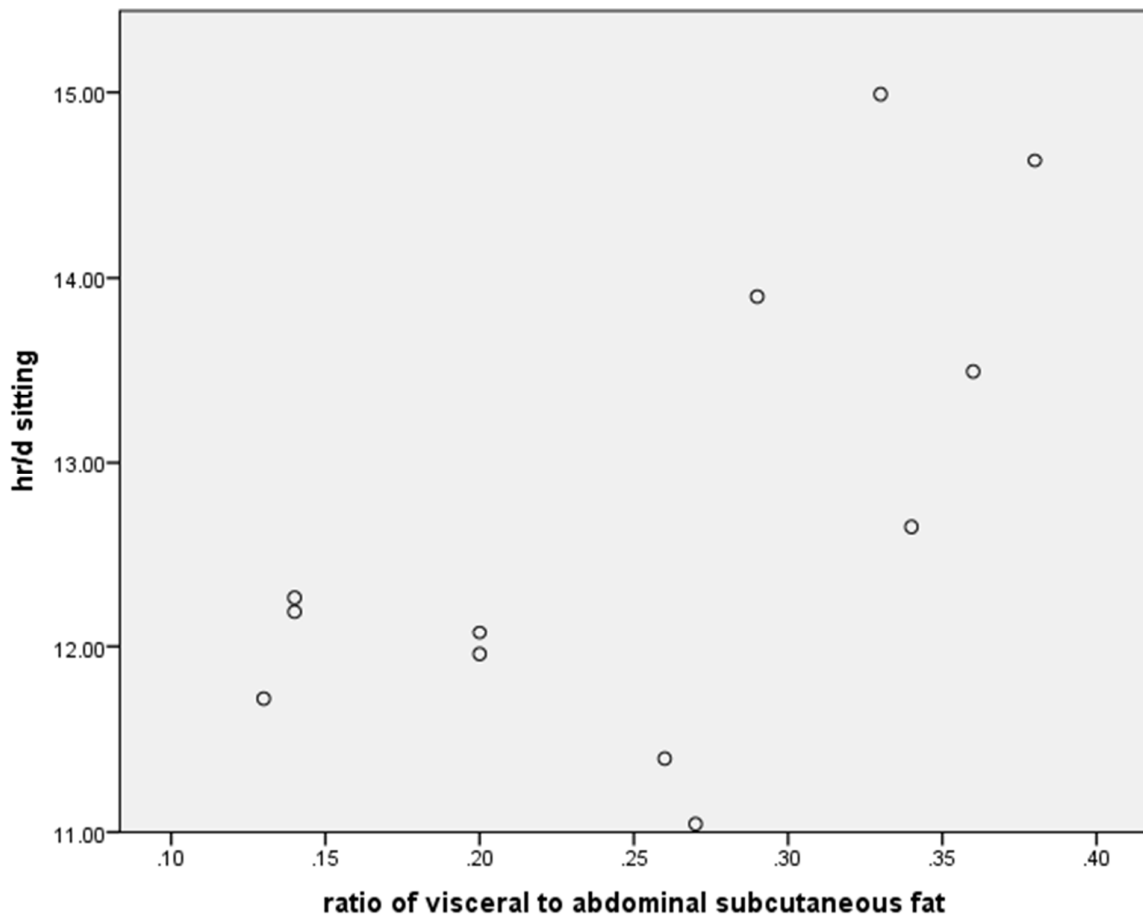


Figure 4. Scatter plot of sitting time against Visceral/subcutaneous abdominal fat ratio.



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The association between objectively measured sitting and standing with body composition: A pilot study using magnetic resonance imaging

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**The association between objectively measured sitting and standing with
body composition: A pilot study using magnetic resonance imaging**

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Word count: 2337

Key words:

Physical activity, sedentary behaviour, adiposity, magnetic resonance imaging

ABSTRACT

Objective: To investigate the association between objectively measured sitting and standing, using a postural allocation technique, with MRI assessed body composition.

Design: The present study was a cross-sectional pilot study.

Setting: Participants were examined at one centre located in London, UK.

Participants: Normal weight Caucasian females (30.9 ± 6.1 yrs; BMI, 22.9 ± 3.4 kg/m²) with desk bound occupations were recruited to minimise variability in body composition outcomes. A convenience sample of 12 females were recruited in January 2014 from University College London

Outcome measures: For each participant a number of body composition variables were attained from a single whole-body magnetic resonance imaging session. Main outcome variables included: total and liver adiposity, visceral/subcutaneous fat ratio, and body mass index. Main exposure variables included: average sitting time, standing: sitting ratio, and step count. Pearson Correlations were carried out to examine associations between different activity categories and body composition variables.

Results: There were significant correlations between average daily sitting and liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively); standing: sitting ratio was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.53$ and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively).

Conclusion: This pilot study has provided preliminary evidence of relationships between objectively measured sitting and standing and precise measures of body composition.

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62 **Article Summary: strengths and limitations of this study**

- 63 • This is the first study to show an association between objectively measured sitting and
64 standing, using a postural allocation technique, with MRI assessed body composition.
- 65 • The data collection protocol and tools used within this pilot study are feasible and can be
66 implemented into subsequent experimental trials.
- 67 • It was not feasible to make multiple statistical adjustments in our analyses owing to the
68 small sample size.

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INTRODUCTION

There is a growing body of literature that suggests sedentary behaviours – defined as any waking behaviour characterised by energy expenditure below 1.5 metabolic equivalents while in a sitting or reclined posture – are associated with higher risk of cardiovascular disease (CVD) and mortality, after statistical adjustment for moderate-to-vigorous intensity physical activities (MVPA; e.g. brisk walking).[1] This has large public health relevance in light of objective data from general adult population studies in the USA and Great Britain that show on average adults spend approximately 60 to 70% of their waking hours in sedentary behaviours.[2] Indeed, westernised society is geared towards promoting sedentary lifestyles (i.e. screen based entertainment, motorised transport etc.), thus, developing strategies to combat sedentary behaviour are crucial.

Such a strategy might not necessarily involve exercise of moderate or vigorous intensity, as interventions to increase exercise levels have proved challenging.[3, 4] Instead, given the barriers to structured exercise (e.g. motivation, cost, access and time etc.), we might consider more subtle lifestyle approaches that are primarily designed to displace sedentary behaviour (i.e. sitting) with forms of lighter intensity (incidental) activity (e.g. standing). If lifestyle population activity patterns can be shifted from predominantly sedentary to the next lowest physical activity (PA) category (standing), this may have public health benefit given the low proportion of individuals meeting current PA guidelines.

To date, limited epidemiological evidence has been generated on the associations between light intensity activity and health. This is partially owing to measurement issues; self-reported PA questionnaires are designed to capture MVPA and there are technical limitations in differentiating between sitting, standing and other forms of light activity when interpreting objective activity data. The most commonly used accelerometer, the Actigraph, quantifies time spent in different intensities of activity by summing time above and below specified count thresholds. This method works reasonably well for identifying MVPA, but is less accurate for distinguishing *between* sedentary and light activity (i.e. between sitting and standing).[5] Thus, methods that employ postural allocation may be more reliable, which have only recently become readily available.

Some experimental evidence is beginning to emerge in this area. For example, one study manipulated sitting time and PA over one day under free living conditions. The results indicated that replacing sitting with longer periods of light activity was more beneficial for metabolic health than one hour of vigorous exercise despite equivalent daily energy expenditure in each treatment group.[6] In a laboratory controlled trial conducted over an 8 hour period, interrupting sitting time

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every 20 mins with short 2-min bouts of light- or moderate intensity walking was shown to lower postprandial glucose and insulin levels in overweight/ obese adults.[7] In another study, using continuously monitored capillary blood glucose, there was a 43% reduction in blood glucose excursion during an afternoon (185 minutes) of standing compared with sitting in desk-based workers.[8] In a pilot study replacing sitting workstations with sit-stand workstations employees reduced sitting time by 137 min/d and increases in HDL-cholesterol were observed at 3 months follow-up.[9] However, the biological mechanisms underlying these effects still remain unclear, although increased muscle activation during standing could be an important underlying mechanism, for example, by increasing skeletal muscle metabolism. Replacing a sitting workstation with a standing workstation was shown to increase daily energy expenditure,[8] thus the longer term benefits might also include reductions in total, visceral, and liver adiposity. A reduction in total and visceral adiposity is known to have a favourable impact on a range of CVD risk factors including inflammatory markers, lipids, and glycaemic control.[10] Liver adiposity is of particular interest as it has been linked to metabolic risk and worsening insulin resistance.[11] Several exercise training studies have demonstrated reductions in visceral adiposity in the absence of weight loss.[12] However, the relationship between light PA (standing) and total, visceral, and liver adiposity has yet to be investigated using precise imaging techniques.

Further research is needed to aid in the understanding of the relationships between objectively measuring sitting and standing, using an objective postural allocation technique, and measures of total, visceral and liver adiposity, using precise imaging techniques. This will contribute to the small but growing body of literature that aims to inform policy and intervention on the health benefits of displacing sitting with standing.

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AIM

In the present study, several contemporary methods were used, including an objective postural allocation technique in combination with magnetic resonance imaging (MRI), to aid in the understanding of the relationships between sitting/ standing and body composition. This data collection was primarily designed to inform a large experimental trial that will investigate the impact of displacing sitting with standing on total, visceral, and liver adiposity. This pilot data will (i) inform the underlying rationale of the trial by producing evidence, if it exists, of relationships between objective measures of sitting and standing and body composition, and (ii) generate an effect size on which to base sample size calculations to inform the main trial.

METHODS

Design, participants and sample size

This cross-sectional pilot study was carried out in 12 healthy Caucasian females. The sample size for this pilot was based on previous published work, which has shown that significant differences in body composition could be readily observed in cross-sectional studies of 10 or less volunteers.[13] Normal weight females with desk bound occupations were selected from a larger cohort to minimise variability in age, weight and overall anthropometry. A convenience sample was recruited in January 2014 from University College London. We randomly invited 12 females who met our criteria to take part in the study. All females invited agreed to take part. One week prior to data collection trained research staff met with the participants to administer the participant information sheets and explain the study protocol.

Measures of adiposity

For each participant, a range of body composition variables were attained from a single whole-body MRI session lasting approximately 45 mins. For the purpose of the present study we defined our main outcomes as *a priori*, which included body mass index (BMI), total litres of body adiposity (L), liver adiposity (%), and visceral/subcutaneous abdominal fat ratio. Whole-body MR images and liver adiposity (%) were obtained on a 1.5 T Phillips Achieva scanner (Philips Medical Systems, Best, The Netherlands) as previously described.[14] Each participant was asked not to participate in strenuous exercise or drink alcohol 24 hours before their scan. Each participant was also requested not to eat and only to drink water from 20:00 on the evening before their scan until the scan was completed. Trained research staff measured participants' height and weight from which BMI was calculated in kg/m².

Free Living Activity

Immediately after the MRI scan, an ActivPal accelerometer/ inclinometer device (<http://www.paltechnologies.com/>) was attached to the participant's thigh mid-way between their right hip and knee and covered with waterproof Tegaderm dressing. The ActivPal classifies an individual's free living activity into periods spent sitting, standing and walking, which it has been

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181 validated for [15]. The ActivPals inclinometer and unique positioning on the thigh allows the device
182 to distinguish between sitting and standing using proprietary algorithms, which previous objective
183 physical activity monitors have been unable to do. The device was worn all day every day
184 (participants were instructed to wear the device during sleep and bathing) for seven full consecutive
185 days. Bespoke software provided by Paltech was used to categorise activity periods into sitting/lying,
186 standing, and stepping, in addition to providing average daily step count. The data are presented as
187 average daily waking time in hours per day (classified as 07:00 to 23:59) spent, sitting, standing, and
188 stepping.

189
190 **Ethics**

191 Written informed consent was obtained from all subjects, and the protocol was approved by the
192 Hammersmith Research Ethics Committee (ref nos: 07/Q0411/19 and 06/Q0411/173).

193
194 **Analysis**

195 Characteristics of the study population, average daily time spent sitting, standing and walking, and
196 the main body composition outcomes were summarised using descriptive statistics. We performed
197 Pearson Correlations to examine associations between different activity categories and the body
198 composition data. For illustrative purposes we also created a standing: sitting ratio and derived two
199 groups based on the median split (high and low). Independent T-tests were performed to compare
200 the main body composition outcomes between groups of high and low standing: sitting ratio. We
201 extracted the partial R² statistic from the correlation between sitting time and liver adiposity to
202 inform a power calculation to provide a sample size for the main trial.

203
204 **Results**

205 Of the 12 females who took part all provided valid MRI and ActivPal data. The volunteers had a
206 mean age of 30.9 ± 6.1 yrs, a mean BMI of 22.9 ± 3.4, and achieved an average of 9993 ± 5146 steps
207 a day (Table 1 contains all descriptive statistics for the study sample). On average participants spent
208 12.7 ± 1.3 hours a day sitting, 3.2 ± 0.9 hours a day standing, 1.8 ± 0.8 hours a day stepping and the
209 remainder in sleep.

Table 1: Descriptive statistics of study sample

Variable	Mean/SD	Range
Age	30.9 ±6.1	24 to 45
BMI (kg/m ²)	22.9 ±3.4	18.1 to 28.1
Total body adipose tissue (L)	24.1 ±9.9	13.2 to 44.4
Liver adiposity (%)	0.52 ±0.73	0.12 to 2.56
Visceral/subcutaneous abdominal fat ratio	0.25 ±0.09	0.13 to 0.38
Average sitting time (hr/d)	12.7 ±1.3	11.0 to 15.0
Average standing time (hr/d)	3.2 ±0.9	1.4 to 4.4
Average stepping (hr/d)	1.8 ±0.8	0.6 to 3.1
Average daily step count	9,993 ±5,146	2,918 to 19,995
Average daily energy expenditure (MET-hr)	24.4 ±2.3	22.6 to 30.2

n=12

There were significant correlations between average daily sitting and liver adiposity and visceral/subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively; see supplementary file one for scatter plots between sitting against body composition outcomes); standing: sitting ratio was moderately correlated with liver adiposity and visceral/subcutaneous abdominal fat ratio ($r=-0.53$ and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total adiposity and visceral/subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively; Table 2). Scatter plots of these associations are presented as supplementary material (see supplementary data; Figures S1-4). We observed weak associations between all activity categories with BMI. Figure One presents comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes. A higher ratio of standing: sitting was consistently associated with lower levels of adiposity. Since the observed associations with sitting may have been influenced by vigorous exercise, we performed sensitivity analyses that removed two participants recording over 15,000 steps (indicative of vigorous exercise). In these analyses ($n=10$) the associations between sitting and adiposity remained largely unchanged; average daily sitting remained associated with liver adiposity ($r=0.65$, $p=0.043$) and visceral/subcutaneous abdominal fat ratio ($r=0.73$, $p=0.017$).

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Table 2: Correlations between ActivPal and MRI measures

	BMI	Total adiposity	Liver fat	Visceral/subcutaneous abdominal fat ratio
Sitting	-0.09	0.10	0.66*	0.64*
Stand: sit ratio	0.24	0.08	-0.53†	-0.45
Av Step-count	-0.22	-0.46	-0.45	-0.51†

237 n=12; Data are Pearson correlations (r). * $p<0.05$; † $p<0.1$

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238 Power Calculation

239 A power calculation was carried out in G-Power to provide a sample size for the main trial. The
240 calculation was based on the correlation between sitting time and liver fat: per 1hr/d sitting was
241 associated with 0.48 [SE, 0.17] unit increase in liver fat [Partial R²= 0.43]. In G-power this equates to
242 an effect size f²= 0.75, and suggests that a sample size of 20 per group would provide us with 95%
243 power at 5% significance level (two-tailed) to detect differences.

245 DISCUSSION

246 The aim of the present study was to investigate the association between objectively measured
247 sitting and standing, using a postural allocation technique (an accelerometer/ inclinometer attached
248 to the participant's thigh mid-way between the hip and the knee), with MRI assessed body
249 composition. Average daily sitting time was associated with liver adiposity and visceral/
250 subcutaneous abdominal fat ratio. Previous studies have attempted to investigate these
251 associations, but without the benefit of the existing gold standard techniques for body composition
252 or full postural allocation measurements. In a recent study using computed tomography, self-
253 reported leisure time sitting was associated with pericardial fat, but not with any other fat
254 depots.[16] We have previously reported associations between objectively assessed sedentary time
255 (Actigraph) and pericardial fat although the relationship did not persist after adjusting for MVPA.[17]
256 Numerous studies have been carried out to investigate the relationship between sedentary time and
257 BMI in adults and found mixed results. For example, one study carried out in a sample of 881 adults
258 residing in Australia found no significant relationship between change in participant-reported TV
259 viewing time and change in BMI, although a cross-sectional association was found between TV
260 viewing time and BMI at baseline, in females only.[18] In another study carried out in a sample of
261 3127 adults residing in Southern France, participant-reported TV viewing time was associated with
262 BMI in both sexes.[19] In the Whitehall II prospective study, BMI predicted sitting time at follow-up
263 but the converse was not found.[20] Conflicting findings may be partially explained by the fact that
264 BMI is a poor indicator of adiposity. Moreover, participants may not be able to recall TV viewing
265 time accurately and TV viewing time may be a poor indicator of total sitting.

266 In comparison to previous research the present study used precise objective measures of both
267 sitting time and body composition. Interestingly, a higher ratio of standing: sitting was associated
268 with lower levels of total, and liver adiposity, and visceral/ subcutaneous fat ratio, providing
269 preliminary cross-sectional evidence of the potential influence of light PA (standing) on body

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270 composition. These findings, although using a more proximal outcome, support previous literature
271 that has found self-reported standing time is inversely related to CVD mortality, in adults.[21]

272 The present pilot study found weak associations between all activity categories and BMI. BMI is a
273 poor measure of adiposity in comparison to MRI since it cannot distinguish between visceral and
274 subcutaneous fat depots. Since visceral and ectopic fat are thought to be more detrimental to health
275 than subcutaneous,[10,11] it is important to distinguish between different types of fat. Furthermore,
276 the numerator in the BMI calculation “total body weight” does not distinguish between lean and fat
277 mass. Therefore, an individual with high levels of lean mass may be classified as having a high BMI;
278 whereas an individual who is of normal weight but has excess body fat may be classified as having a
279 normal BMI. This might partly explain why several exercise training studies have demonstrated
280 reductions in visceral adiposity in the absence of weight loss.[12]

281 The *data collection* protocol and tools used within this small pilot study are feasible and can be
282 implemented into the subsequent experimental trial; a 100% response rate was achieved and no
283 participant dropped out of the study. Moreover, all participants provided a full ActivPal dataset
284 (seven complete days) and adhered to the wear protocol. However, it should be noted that the
285 subsequent experimental trial will require two identical data collection sessions to assess the impact
286 of displacing sitting with standing on body composition.

287 It was not feasible to make multiple statistical adjustments in our analyses owing to the small
288 sample size, thus associations between sitting and adiposity may have been confounded by vigorous
289 exercise. However, we selected a homogenous sample and the removal of two highly active
290 participants in our sample did not change the results. Given the cross-sectional nature of this pilot
291 study the direction of the observed associations remains unknown. Moreover, the
292 representativeness of the findings are limited, owing to the small sample size of healthy Caucasian
293 females residing in London. However, the aim of this pilot study was to provide novel data to
294 support the underlying rationale and generate a sample size for a subsequent experimental trial.

295 Inclusion criteria for the experimental trial, that this pilot study was carried out to inform, will be
296 overweight/ obesity. We will use a number of biomedical outcomes in the main trial including body
297 composition (MRI), and biochemical risk markers (lipids, inflammatory markers, glucose).

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CONCLUSION

This pilot study has provided preliminary evidence of the strong relationships between objectively measured sitting and standing (an accelerometer/ inclinometer attached to the participant's thigh mid-way between the hip and the knee) and precise measures of body composition.

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328 study design; in the collection, analysis and interpretation of data; in writing of the report; or in the
329 decision to submit the paper for publication.

330 **Authors’ contributions**

331 LS and MH designed the study. All authors contributed to development of the study protocol. LS and
332 MH drafted the manuscript. LT and JB assisted in drafting the manuscript. All authors read and
333 approved the final manuscript

334 **Competing interests**

335 The authors declare that they have no competing interests.

336 **Data Sharing Statement**

337 No additional data is available

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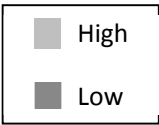
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Figure one Legend



A, Body mass index; B, Total adipose fat (litres); C, liver fat (%); D, visceral/subcutaneous abdominal fat ratio; n=12.

The high: low cut point was ≥ 0.27

Supplementary Figure Legends

Figure 1. Scatter plot of sitting time against body mass index.

Figure 2. Scatter plot of sitting time against total adiposity.

Figure 3. Scatter plot of sitting time against liver fat.

Figure 4. Scatter plot of sitting time against Visceral/subcutaneous abdominal fat ratio.

The association between objectively measured sitting and standing with body composition: A pilot study using magnetic resonance imaging

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Word count: 2337

Key words:

Physical activity, sedentary behaviour, adiposity, magnetic resonance imaging

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30 **ABSTRACT**

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32 **Objective:** To investigate the association between objectively measured sitting and standing, using a
33 postural allocation technique, with MRI assessed body composition.

34

35 **Design:** The present study was a cross-sectional pilot study.

36

37 **Setting:** Participants were examined at one centre located in London, UK.

38

39 **Participants:** Normal weight Caucasian females (30.9 ± 6.1 yrs; BMI, 22.9 ± 3.4 kg/m²) with desk
40 bound occupations were recruited to minimise variability in body composition outcomes. A
41 convenience sample of 12 females were recruited in January 2014 from University College London

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43 **Outcome measures:** For each participant a number of body composition variables were attained
44 from a single whole-body magnetic resonance imaging session. Main outcome variables included:
45 total and liver adiposity, visceral/subcutaneous fat ratio, and body mass index. Main exposure
46 variables included: average sitting time, standing: sitting ratio, and step count. Pearson Correlations
47 were carried out to examine associations between different activity categories and body
48 composition variables.

49

50 **Results:** There were significant correlations between average daily sitting and liver adiposity and
51 visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively); standing: sitting ratio
52 was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-$
53 0.53 and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total
54 adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively).

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56 **Conclusion:** This pilot study has provided preliminary evidence of relationships between objectively
57 measured sitting and standing and precise measures of body composition.

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Article Summary: strengths and limitations of this study

- This is the first study to show an association between objectively measured sitting and standing, using a postural allocation technique, with MRI assessed body composition.
- The data collection protocol and tools used within this pilot study are feasible and can be implemented into subsequent experimental trials.
- It was not feasible to make multiple statistical adjustments in our analyses owing to the small sample size.

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87 **INTRODUCTION**

88 There is a growing body of literature that suggests sedentary behaviours – defined as any waking
89 behaviour characterised by energy expenditure below 1.5 metabolic equivalents while in a sitting or
90 reclined posture – are associated with higher risk of cardiovascular disease (CVD) and mortality, after
91 statistical adjustment for moderate-to-vigorous intensity physical activities (MVPA; e.g. brisk
92 walking).[1] This has large public health relevance in light of objective data from general adult
93 population studies in the USA and Great Britain that show on average adults spend approximately 60
94 to 70% of their waking hours in sedentary behaviours.[2] Indeed, westernised society is geared
95 towards promoting sedentary lifestyles (i.e. screen based entertainment, motorised transport etc.),
96 thus, developing strategies to combat sedentary behaviour are crucial.

97 Such a strategy might not necessarily involve exercise of moderate or vigorous intensity, as
98 interventions to increase exercise levels have proved challenging.[3, 4] Instead, given the barriers to
99 structured exercise (e.g. motivation, cost, access and time etc.), we might consider more subtle
100 lifestyle approaches that are primarily designed to displace sedentary behaviour (i.e. sitting) with
101 forms of lighter intensity (incidental) activity (e.g. standing). If lifestyle population activity patterns
102 can be shifted from predominantly sedentary to the next lowest physical activity (PA) category
103 (standing), this may have public health benefit given the low proportion of individuals meeting
104 current PA guidelines.

105 To date, limited epidemiological evidence has been generated on the associations between light
106 intensity activity and health. This is partially owing to measurement issues; self-reported PA
107 questionnaires are designed to capture MVPA and there are technical limitations in differentiating
108 between sitting, standing and other forms of light activity when interpreting objective activity data.
109 The most commonly used accelerometer, the Actigraph, quantifies time spent in different intensities
110 of activity by summing time above and below specified count thresholds. This method works
111 reasonably well for identifying MVPA, but is less accurate for distinguishing *between* sedentary and
112 light activity (i.e. between sitting and standing).[5] Thus, methods that employ postural allocation
113 may be more reliable, which have only recently become readily available.

114 Some experimental evidence is beginning to emerge in this area. For example, one study
115 manipulated sitting time and PA over one day under free living conditions. The results indicated that
116 replacing sitting with longer periods of light activity was more beneficial for metabolic health than
117 one hour of vigorous exercise despite equivalent daily energy expenditure in each treatment
118 group.[6] In a laboratory controlled trial conducted over an 8 hour period, interrupting sitting time

every 20 mins with short 2-min bouts of light- or moderate intensity walking was shown to lower postprandial glucose and insulin levels in overweight/ obese adults.[7] In another study, using continuously monitored capillary blood glucose, there was a 43% reduction in blood glucose excursion during an afternoon (185 minutes) of standing compared with sitting in desk-based workers.[8] In a pilot study replacing sitting workstations with sit-stand workstations employees reduced sitting time by 137 min/d and increases in HDL-cholesterol were observed at 3 months follow-up.[9] However, the biological mechanisms underlying these effects still remain unclear, although increased muscle activation during standing could be an important underlying mechanism, for example, by increasing skeletal muscle metabolism. Replacing a sitting workstation with a standing workstation was shown to increase daily energy expenditure,[8] thus the longer term benefits might also include reductions in total, visceral, and liver adiposity. A reduction in total and visceral adiposity is known to have a favourable impact on a range of CVD risk factors including inflammatory markers, lipids, and glycaemic control.[10] Liver adiposity is of particular interest as it has been linked to metabolic risk and worsening insulin resistance.[11] Several exercise training studies have demonstrated reductions in visceral adiposity in the absence of weight loss.[12] However, the relationship between light PA (standing) and total, visceral, and liver adiposity has yet to be investigated using precise imaging techniques.

Further research is needed to aid in the understanding of the relationships between objectively measuring sitting and standing, using an objective postural allocation technique, and measures of total, visceral and liver adiposity, using precise imaging techniques. This will contribute to the small but growing body of literature that aims to inform policy and intervention on the health benefits of displacing sitting with standing.

141

142 AIM

143 In the present study, several contemporary methods were used, including an objective postural
144 allocation technique in combination with magnetic resonance imaging (MRI), to aid in the
145 understanding of the relationships between sitting/ standing and body composition. This data
146 collection was primarily designed to inform a large experimental trial that will investigate the impact
147 of displacing sitting with standing on total, visceral, and liver adiposity. This pilot data will (i) inform
148 the underlying rationale of the trial by producing evidence, if it exists, of relationships between
149 objective measures of sitting and standing and body composition, and (ii) generate an effect size on
150 which to base sample size calculations to inform the main trial.

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151 **METHODS**

152

153 **Design, participants and sample size**

154 This cross-sectional pilot study was carried out in 12 healthy Caucasian females. The sample size for
155 this pilot was based on previous published work, which has shown that significant differences in
156 body composition could be readily observed in cross-sectional studies of 10 or less volunteers.[13]
157 Normal weight females with desk bound occupations were selected from a larger cohort to minimise
158 variability in age, weight and overall anthropometry. A convenience sample was recruited in January
159 2014 from University College London. We randomly invited 12 females who met our criteria to take
160 part in the study. All females invited agreed to take part. One week prior to data collection trained
161 research staff met with the participants to administer the participant information sheets and explain
162 the study protocol.

163

164 **Measures of adiposity**

165 For each participant, a range of body composition variables were attained from a single whole-body
166 MRI session lasting approximately 45 mins. For the purpose of the present study we defined our
167 main outcomes as *a priori*, which included body mass index (BMI), total litres of body adiposity (L),
168 liver adiposity (%), and visceral/subcutaneous abdominal fat ratio. Whole-body MR images and liver
169 adiposity (%) were obtained on a 1.5 T Phillips Achieva scanner (Philips Medical Systems, Best, The
170 Netherlands) as previously described.[14] Each participant was asked not to participate in strenuous
171 exercise or drink alcohol 24 hours before their scan. Each participant was also requested not to eat
172 and only to drink water from 20:00 on the evening before their scan until the scan was completed.
173 Trained research staff measured participants' height and weight from which BMI was calculated in
174 kg/m².

175

176 **Free Living Activity**

177 Immediately after the MRI scan, an ActivPal accelerometer/ inclinometer device
178 (<http://www.paltechnologies.com/>) was attached to the participant's thigh mid-way between their
179 right hip and knee and covered with waterproof Tegaderm dressing. The ActivPal classifies an
180 individual's free living activity into periods spent sitting, standing and walking, which it has been

validated for [15]. The ActivPals inclinometer and unique positioning on the thigh allows the device to distinguish between sitting and standing using proprietary algorithms, which previous objective physical activity monitors have been unable to do. The device was worn all day every day (participants were instructed to wear the device during sleep and bathing) for seven full consecutive days. Bespoke software provided by Paltech was used to categorise activity periods into sitting/lying, standing, and stepping, in addition to providing average daily step count. The data are presented as average daily waking time in hours per day (classified as 07:00 to 23:59) spent, sitting, standing, and stepping.

Ethics

Written informed consent was obtained from all subjects, and the protocol was approved by the Hammersmith Research Ethics Committee (ref nos: 07/Q0411/19 and 06/Q0411/173).

Analysis

Characteristics of the study population, average daily time spent sitting, standing and walking, and the main body composition outcomes were summarised using descriptive statistics. We performed Pearson Correlations to examine associations between different activity categories and the body composition data. For illustrative purposes we also created a standing: sitting ratio and derived two groups based on the median split (high and low). Independent T-tests were performed to compare the main body composition outcomes between groups of high and low standing: sitting ratio. We extracted the partial R^2 statistic from the correlation between sitting time and liver adiposity to inform a power calculation to provide a sample size for the main trial.

Results

Of the 12 females who took part all provided valid MRI and ActivPal data. The volunteers had a mean age of 30.9 ± 6.1 yrs, a mean BMI of 22.9 ± 3.4 , and achieved an average of 9993 ± 5146 steps a day (Table 1 contains all descriptive statistics for the study sample). On average participants spent 12.7 ± 1.3 hours a day sitting, 3.2 ± 0.9 hours a day standing, 1.8 ± 0.8 hours a day stepping and the remainder in sleep.

Table 1: Descriptive statistics of study sample

Variable	Mean/SD	Range
Age	30.9 ±6.1	24 to 45
BMI (kg/m ²)	22.9 ±3.4	18.1 to 28.1
Total body adipose tissue (L)	24.1 ±9.9	13.2 to 44.4
Liver adiposity (%)	0.52 ±0.73	0.12 to 2.56
Visceral/subcutaneous abdominal fat ratio	0.25 ±0.09	0.13 to 0.38
Average sitting time (hr/d)	12.7 ±1.3	11.0 to 15.0
Average standing time (hr/d)	3.2 ±0.9	1.4 to 4.4
Average stepping (hr/d)	1.8 ±0.8	0.6 to 3.1
Average daily step count	9,993 ±5,146	2,918 to 19,995
Average daily energy expenditure (MET-hr)	24.4 ±2.3	22.6 to 30.2

n=12

There were significant correlations between average daily sitting and liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=0.66$ and $r=0.64$, respectively; see supplementary file one for scatter plots between sitting against body composition outcomes); standing: sitting ratio was moderately correlated with liver adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.53$ and $r=-0.45$); average daily step count was moderately correlated with liver adiposity, total adiposity and visceral/ subcutaneous abdominal fat ratio ($r=-0.45$, $r=-0.46$, and $r=-0.51$, respectively; Table 2). Scatter plots of these associations are presented as supplementary material (see supplementary data; Figures S1-4). We observed weak associations between all activity categories with BMI. Figure One presents comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes. A higher ratio of standing: sitting was consistently associated with lower levels of adiposity. Since the observed associations with sitting may have been influenced by vigorous exercise, we performed sensitivity analyses that removed two participants recording over 15,000 steps (indicative of vigorous exercise). In these analyses ($n=10$) the associations between sitting and adiposity remained largely unchanged; average daily sitting remained associated with liver adiposity ($r=0.65$, $p=0.043$) and visceral/ subcutaneous abdominal fat ratio ($r=0.73$, $p=0.017$).

Table 2: Correlations between ActivPal and MRI measures

	BMI	Total adiposity	Liver fat	Visceral/subcutaneous abdominal fat ratio
Sitting	-0.09	0.10	0.66*	0.64*
Stand: sit ratio	0.24	0.08	-0.53†	-0.45
Av Step-count	-0.22	-0.46	-0.45	-0.51†

n=12; Data are Pearson correlations (r). * $p<0.05$; † $p<0.1$

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238 **Power Calculation**

239 A power calculation was carried out in G-Power to provide a sample size for the main trial. The
240 calculation was based on the correlation between sitting time and liver fat: per 1hr/d sitting was
241 associated with 0.48 [SE, 0.17] unit increase in liver fat [Partial R²= 0.43]. In G-power this equates to
242 an effect size f²= 0.75, and suggests that a sample size of 20 per group would provide us with 95%
243 power at 5% significance level (two-tailed) to detect differences.

244

245 **DISCUSSION**

246 The aim of the present study was to investigate the association between objectively measured
247 sitting and standing, using a postural allocation technique (an accelerometer/ inclinometer attached
248 to the participant's thigh mid-way between the hip and the knee), with MRI assessed body
249 composition. Average daily sitting time was associated with liver adiposity and visceral/
250 subcutaneous abdominal fat ratio. Previous studies have attempted to investigate these
251 associations, but without the benefit of the existing gold standard techniques for body composition
252 or full postural allocation measurements. In a recent study using computed tomography, self-
253 reported leisure time sitting was associated with pericardial fat, but not with any other fat
254 depots.[16] We have previously reported associations between objectively assessed sedentary time
255 (Actigraph) and pericardial fat although the relationship did not persist after adjusting for MVPA.[17]
256 Numerous studies have been carried out to investigate the relationship between sedentary time and
257 BMI in adults and found mixed results. For example, one study carried out in a sample of 881 adults
258 residing in Australia found no significant relationship between change in participant-reported TV
259 viewing time and change in BMI, although a cross-sectional association was found between TV
260 viewing time and BMI at baseline, in females only.[18] In another study carried out in a sample of
261 3127 adults residing in Southern France, participant-reported TV viewing time was associated with
262 BMI in both sexes.[19] In the Whitehall II prospective study, BMI predicted sitting time at follow-up
263 but the converse was not found.[20] Conflicting findings may be partially explained by the fact that
264 BMI is a poor indicator of adiposity. Moreover, participants may not be able to recall TV viewing
265 time accurately and TV viewing time may be a poor indicator of total sitting.

266 In comparison to previous research the present study used precise objective measures of both
267 sitting time and body composition. Interestingly, a higher ratio of standing: sitting was associated
268 with lower levels of total, and liver adiposity, and visceral/ subcutaneous fat ratio, providing
269 preliminary cross-sectional evidence of the potential influence of light PA (standing) on body

270 composition. These findings, although using a more proximal outcome, support previous literature
271 that has found self-reported standing time is inversely related to CVD mortality, in adults.[21]

272 The present pilot study found weak associations between all activity categories and BMI. BMI is a
273 poor measure of adiposity in comparison to MRI since it cannot distinguish between visceral and
274 subcutaneous fat depots. Since visceral and ectopic fat are thought to be more detrimental to health
275 than subcutaneous,[10,11] it is important to distinguish between different types of fat. Furthermore,
276 the numerator in the BMI calculation “total body weight” does not distinguish between lean and fat
277 mass. Therefore, an individual with high levels of lean mass may be classified as having a high BMI;
278 whereas an individual who is of normal weight but has excess body fat may be classified as having a
279 normal BMI. This might partly explain why several exercise training studies have demonstrated
280 reductions in visceral adiposity in the absence of weight loss.[12]

281 The *data collection* protocol and tools used within this small pilot study are feasible and can be
282 implemented into the subsequent experimental trial; a 100% response rate was achieved and no
283 participant dropped out of the study. Moreover, all participants provided a full ActivPal dataset
284 (seven complete days) and adhered to the wear protocol. However, it should be noted that the
285 subsequent experimental trial will require two identical data collection sessions to assess the impact
286 of displacing sitting with standing on body composition.

287 It was not feasible to make multiple statistical adjustments in our analyses owing to the small
288 sample size, thus associations between sitting and adiposity may have been confounded by vigorous
289 exercise. However, we selected a homogenous sample and the removal of two highly active
290 participants in our sample did not change the results. Given the cross-sectional nature of this pilot
291 study the direction of the observed associations remains unknown. Moreover, the
292 representativeness of the findings are limited, owing to the small sample size of healthy Caucasian
293 females residing in London. However, the aim of this pilot study was to provide novel data to
294 support the underlying rationale and generate a sample size for a subsequent experimental trial.

295 Inclusion criteria for the experimental trial, that this pilot study was carried out to inform, will be
296 overweight/ obesity. We will use a number of biomedical outcomes in the main trial including body
297 composition (MRI), and biochemical risk markers (lipids, inflammatory markers, glucose).

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301 **CONCLUSION**

302 This pilot study has provided preliminary evidence of the strong relationships between objectively
303 measured sitting and standing (an accelerometer/ inclinometer attached to the participant’s thigh
304 mid-way between the hip and the knee) and precise measures of body composition.

305

306 **Competing interests**

307 The authors declare that they have no competing interests.

308

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312 study design; in the collection, analysis and interpretation of data; in writing of the report; or in the
313 decision to submit the paper for publication.

314

315 **Authors’ contributions**

316 LS and MH designed the study. All authors contributed to development of the study protocol. LS and
317 MH drafted the manuscript. LT and JB assisted in drafting the manuscript. All authors read and
318 approved the final manuscript.

319

320 **Figure one Legend**



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324 A, Body mass index; B, Total adipose fat (litres); C, liver fat (%); D, visceral/subcutaneous
325 abdominal fat ratio; n=12.

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327 The high: low cut point was ≥ 0.27

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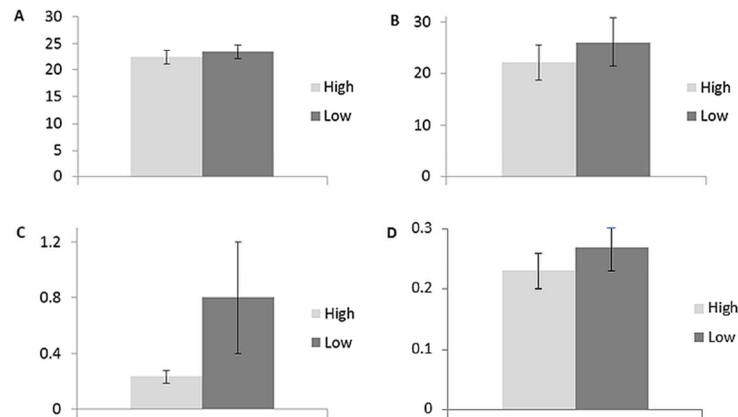
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Figure 1: comparisons between groups of high and low standing: sitting ratio in relation to the main body composition outcomes



A, Body mass index; B, Total adipose fat (litres); C, liver fat (%); D, visceral/subcutaneous abdominal fat ratio; n=12

The high: low cut point was ≥ 0.27

90x67mm (300 x 300 DPI)

Supplementary data

Figure 1. Scatter plot of sitting time against body mass index.

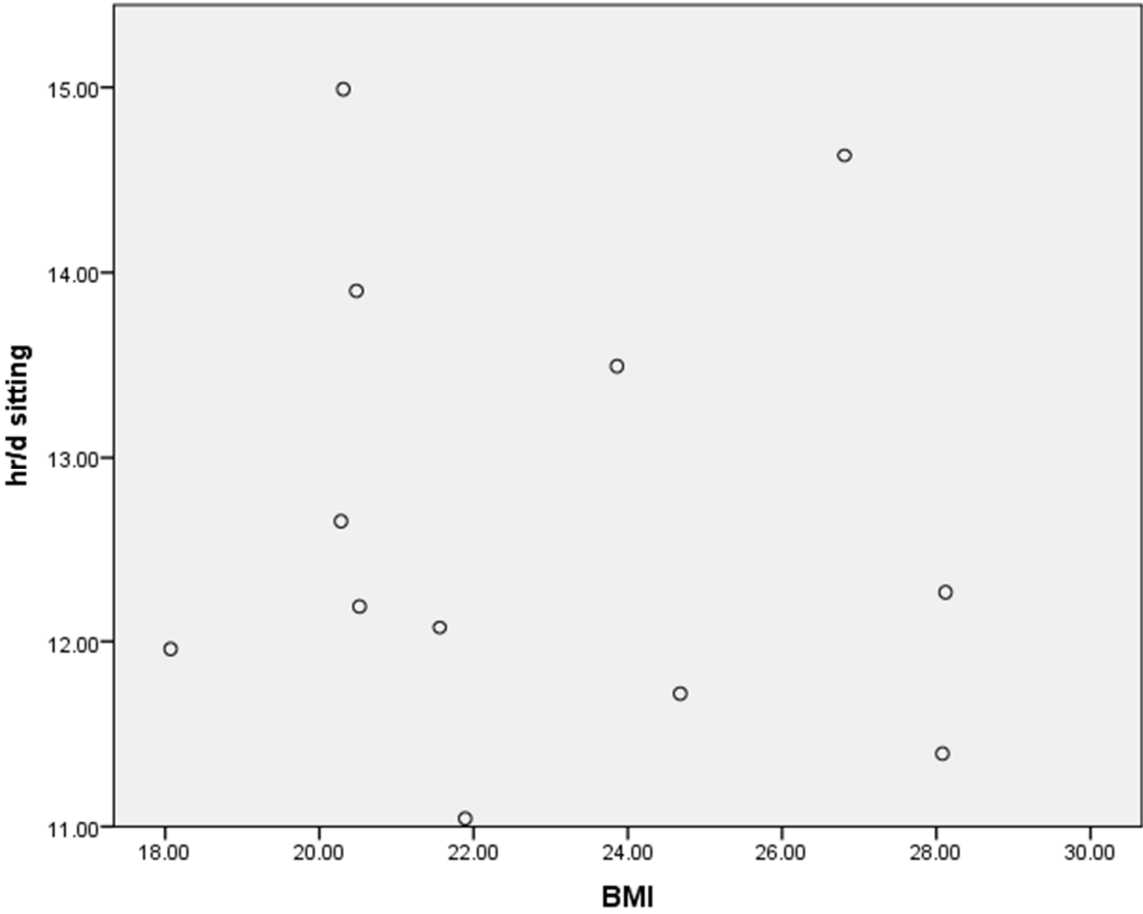


Figure 2. Scatter plot of sitting time against total adiposity.

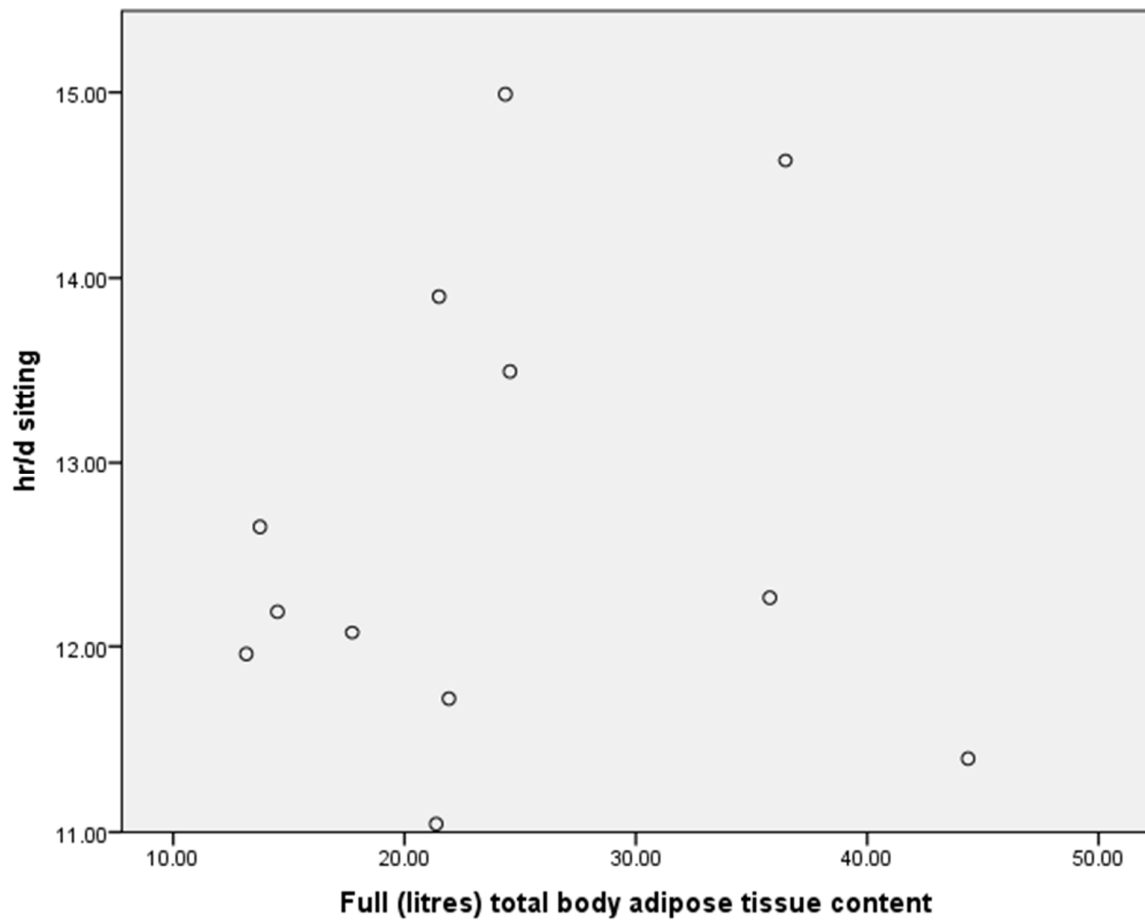


Figure 3. Scatter plot of sitting time against liver fat.

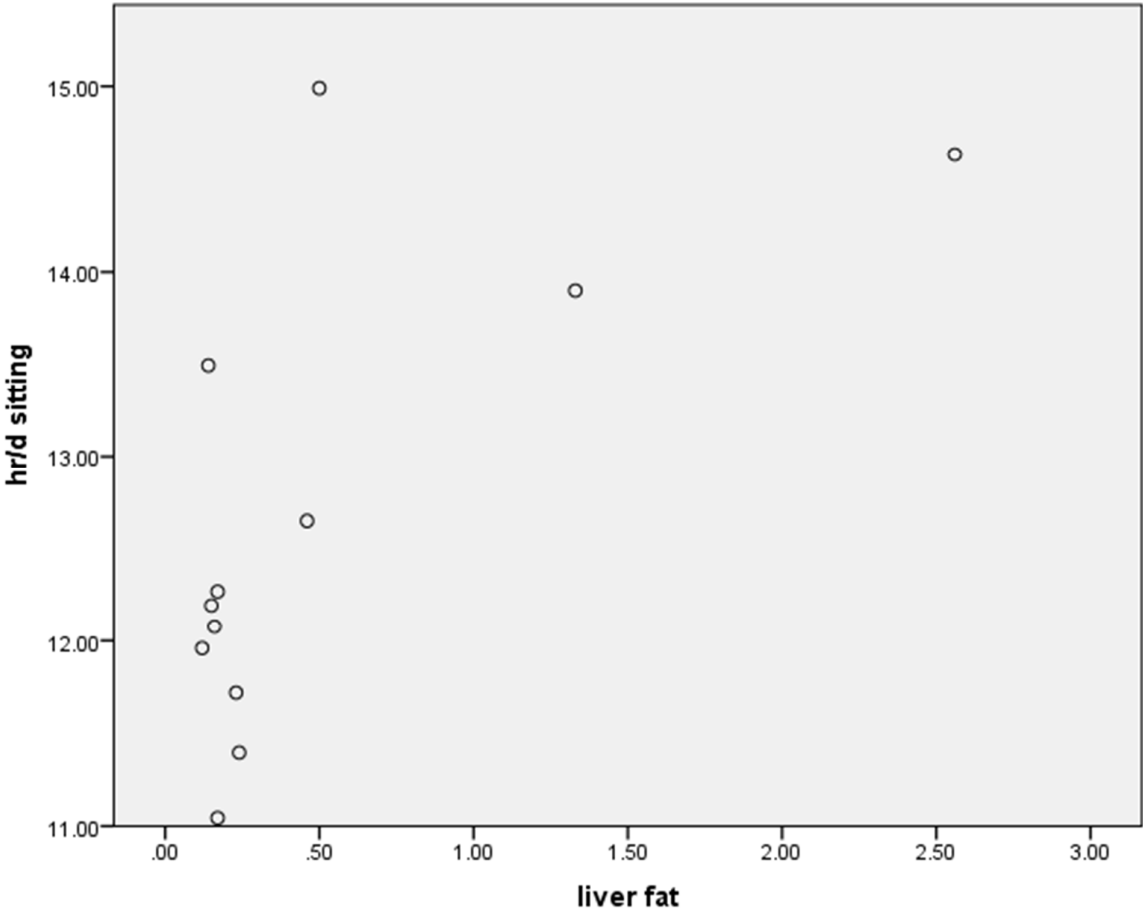


Figure 4. Scatter plot of sitting time against Visceral/subcutaneous abdominal fat ratio.

